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A STUDY OF THE RELATION OF THE BLOOD SUGAR IN PLASMA TO THAT IN THE CORPUSCLES IN NORMAL AND DIABETIC INDIVIDUALS*

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A COMPARISON of the sugar content of the blood plasma with the sugar content of the corpuscles shows, as a rule, a variation in both directions; in other words, the level of the corpuscular sugar may be slightly higher or slightly lower than that of the plasma. In 1923 I undertook a study[1] of this relationship with the material which was then available, and the present work is merely a continuation of this former study carried out with more extensive material. The data given in this paper have been collected from the study of the blood in 193 glucose tolerance tests, 103 of which were made in diabetic, and 90 in nondiabetic patients.

The tests were performed in a routine manner, and were always made while the patient was fasting. A specimen of blood was first taken, after which the patient received 100 gm. of anhydrous glucose dissolved in 250 to 300 c.c. of water to which the juice of one lemon had been added. This was made more palatable by being chilled. In the case of children under fifteen years of age, only 75 gm. of glucose were used. Specimens of blood were again taken one-half hour, one hour, two, three, and four hours after the ingestion of the glucose.

The sugar content of the whole blood, of the corpuscles, and of the plasma was determined in the following manner: The oxalated blood was centrifuged at high speed for fifteen minutes, the plasma was drawn off, and one c.c. of the corpuscles taken for the sugar determination. The corpuscles

*From the Cleveland Clinic.

were not washed. Myers' modification of the Benedict method of blood-sugar estimation was used throughout these tests, the readings being made by means of the Kober colorimeter. In this study the plasma sugar was taken as a standard with which other estimations were compared, and the relative increase or decrease of the sugar was then calculated.

The plasma contains a certain amount of sugar in solution; and since the corpuscles are surrounded by the plasma, the question arises as to whether or not an equilibrium may be reached. On the other hand, the corpuscles are living bodies and consume sugar, just as do the tissues, while the plasma is simply a vehicle to supply energy to the corpuscles in the form of sugar. Consequently, even though an equilibrium might be reached, one would perhaps expect to find the sugar content of the corpuscles lower than that of the plasma, especially if the plasma sugar were higher than normal. If this were the only factor, then the sugar content of the corpuscles should not rise above a certain level; in other words, it should be constant under all conditions. That this is not the case can easily be demonstrated, simply by a comparison of the sugar content of the corpuscles in the blood of a normal individual with that in the blood of a patient with marked hyperglycemia. In the latter case the sugar content of the corpuscles will be high, and one is convinced that the factor of osmosis plays a rôle here, since the red corpuscle cell is quite different in this respect from a muscle cell.

The red corpuscle, which is completely surrounded by the sugar-carrying plasma, absorbs by osmosis enough sugar to supply its own energy plus the amount necessary to establish an equilibrium between the two sides of the membrane. We are dealing here with several inconstant variables: (1) Changes in the sugar content of the plasma, which is constantly giving off sugar to the tissues. (2) The intake of sugar into the blood stream from the stores in the liver, which is also not a strictly constant phenomenon. (3) The utilization by the corpuscles of the sugar within their walls and the consequent decrease in their store of sugar. (4) Osmosis of sugar through the corpuscular membrane, first, to replace that which has been used, and second, to equalize the pressure on the two sides of the membrane.

A comparison of the changes in the sugar content of the corpuscles and of the plasma in Table I (nondiabetics) shows that in general the sugar content of the corpuscles is lower than that of the plasma, and that as the sugar level of the plasma rises, following the ingestion of glucose, the sugar level of the corpuscles does not keep pace with it. As the sugar starts to decrease in the plasma, the corpuscles release their sugar less rapidly and we find a higher sugar level in the corpuscles. This confirms the observations of Gradwohl and Blaivas.[2] An examination of Table II (diabetics) shows conditions somewhat different from those in nondiabetic subjects. Thus, in diabetic individuals, the level of the corpuscular sugar is lower than that of the plasma sugar and only rarely does one find at the end of the test that the sugar content of the corpuscles is higher than that of the plasma. From a comparison of the two tables one gets the impression that in the presence of diabetes the corpuscles do not take in the sugar as rapidly as is normal, and that con-

sequently their sugar content stays for the most part below that of the plasma. Perhaps the lack of insulin may have something to do with this storing of sugar within the corpuscular wall, just as it does in the case of tissue cells. The corpuscles of diabetic individuals, therefore, differ from the corpuscles of normal individuals by their lessened permeability or capacity to take in and to hold sugar.

A study of Tables I and II shows that in 246 estimations of the blood sugar made in nondiabetic individuals, and in 44 in diabetic individuals there was an increase in the corpuscular sugar as compared to the plasma sugar, the average increase in the nondiabetic cases being 17.12 per cent, and in the diabetic cases, 9.95 per cent. On the other hand, in 236 estimations in nondiabetic individuals and in 511 in diabetic individuals, there was a decrease in the corpuscular sugar as compared with the plasma sugar, the average decrease in the nondiabetic cases being 12.20 per cent, and in the diabetic cases, 15.82 per cent. (These comparative data are given in Table III.) We may say, therefore, that among the nondiabetic individuals the ratio of the average increase in the corpuscular sugar over the plasma sugar to the average decrease was as 17.12 to 12.20, while the corresponding ratio among the diabetics was as 9.95 to 15.82. Thus, among nondiabetic individuals the level of the corpuscular sugar was higher than that of the plasma sugar, while among the diabetic subjects the level of the plasma sugar was higher in two-thirds of the estimations. These figures emphasize the fact that in the presence of diabetes the blood corpuscles are, on the whole, much less permeable to sugar than they are normally.

As for cases of severe diabetes, that is, those in which the blood sugar at the beginning of the glucose tolerance test was 250 or above, it is of interest to see whether or not the ratios remain the same. An analysis of 85 blood-sugar examinations in individuals with severe diabetes gave the following results:

Total number of examinations	85
Total decrease	1,427
Average decrease in corpuscular sugar as compared with plasma sugar	16.79 per cent
Increase in corpuscular sugar as compared with plasma sugar	0

The average decrease in the corpuscular sugar as compared with the plasma sugar, therefore, was practically the same as that observed in the total number of observations.

Another attempt to measure the permeability of the red corpuscles directly was made by the following method: The corpuscles were suspended in a solution of 1,000 mg. per 100 c.c. of glucose in N/10 salt solution for two hours. The corpuscles of the nondiabetic individuals were then compared with those of the diabetics (Table IV). It was found that the actual percentage of increase in corpuscular sugar among the nondiabetic individuals

TABLE I

RELATION OF PLASMA SUGAR TO CORPUSCLE SUGAR IN NON-DIABETIC PATIENTS
(Estimations made during glucose tolerance tests)

Hours after ingestion of glucose

	0	½	1	2	3	4	0	½	1	2	3	4	0	½	1	2	3	4	0	½	1	2	3	4	0	½	1	2	3	4
Whole Blood	89	111	97	100	86	83	117	174	151	94	87	90	97	143	183	127	78	82	94	153	123	114	81	82	133	192	139	113	88	96	92	143	111	95	81	90	99	127	108	92	98	89
Plasma	84	113	84	98	79	84	112	187	129	85	74	80	85	146	191	117	78	83	88	130	98	87	74	69	107	187	135	107	70	94	87	141	116	80	70	79	79	126	96	83	97	88
Corpuscles	92	96	79	100	84	82	160	166	123	104	96	96	92	123	164	121	86	86	-	146	116	117	101	91	140	185	147	114	107	110	91	121	99	93	93	-	105	129	100	107	97	-
Corp/Pl incr	-9	-	-	-2	-6	-	-	-	-	-	22	30	6	8	-	-	4	13	24	-	12	19	20	36	38	31	-	8	6	52	17	4	-	-	16	32	-	-	33	-	4	3	-	-
Corp/Pl decr	-	15	7	-	-	-2	2	10	5	-	-	-	-	-	9	14	-	-	-	-	-	-	-	-	-	-	1	-	-	-	-	14	15	-	-	-	-	-	2	-	3	-
Whole Blood	106	161	174	129	109	78	94	182	184	125	95	82	90	151	146	121	89	79	87	124	83	207	118	75	120	156	171	122	107	104	94	160	159	123	73	81	93	178	197	133	81	67
Plasma	89	172	186	129	104	68	87	166	182	120	90	56	89	169	141	105	68	73	88	125	86	197	124	60	115	161	164	117	88	115	94	163	167	131	68	73	86	197	219	132	81	66
Corpuscles	-	150	131	109	103	73	92	141	196	140	113	61	92	162	132	124	69	77	85	120	170	199	129	69	130	162	161	155	133	122	83	142	145	106	50	74	78	146	164	129	58	-
Corp/Pl incr	-	-	-	-	-	-8	6	-	7	17	26	69	3	-	-	24	44	5	-	-	-	1	2	49	13	-	-	32	50	8	-	-	-	-	1	-	-	-	-	13	-
Corp/Pl decr	-	13	30	16	1	-	-	15	-	-	-	-	-	6	6	-	-	-	4	2	9	-	-	-	-	-	2	-	-	-	12	13	14	19	26	-	8	26	25	2	-	-
Whole Blood	104	201	208	146	121	88	98	198	194	136	73	56	99	126	103	76	58	78	98	158	159	111	94	120	107	135	130	135	98	85	105	169	145	122	81	90	109	155	141	105	89	78
Plasma	111	228	217	146	119	87	86	212	190	141	61	49	94	126	113	63	49	68	93	159	152	94	70	116	99	135	121	121	80	71	99	149	170	104	61	80	98	155	136	106	76	63
Corpuscles	104	192	176	125	116	75	94	158	146	152	78	82	95	121	104	74	64	75	110	176	164	116	111	-	106	138	127	136	109	110	98	162	136	114	88	104	111	136	143	97	95	70
Corp/Pl incr	-	-	-	-	-	-	9	-	-	-	28	6	1	-	-	18	30	-7	18	11	8	24	59	-	-	7	2	-	11	36	55	-	2	-	10	44	30	14	-	5	-	24	10
Corp/Pl decr	6	16	19	15	3	14	-	26	23	7	-	-	-	4	8	-	-	-	-	-	-	-	-	-	-	-	3	-	-	-	1	-	20	-	-	-	13	-	7	-	-
Whole Blood	104	207	162	125	85	79	103	154	201	117	69	86	98	121	90	114	84	61	85	138	104	107	62	109	106	115	124	91	68	96	106	185	197	126	89	80	108	150	145	111	58	74
Plasma	83	214	200	113	80	73	94	164	219	118	64	74	90	121	123	127	51	84	105	146	100	91	60	102	97	113	119	93	63	84	94	180	200	117	60	74	92	147	146	70	38	63
Corpuscles	94	167	150	-	78	79	92	136	172	109	58	84	87	104	125	126	58	-	104	159	108	107	66	91	104	116	120	97	73	96	105	161	174	120	85	78	105	150	141	120	-	66
Corp/Pl incr	15	-	-	-	55	5	-	-	-	-	-	15	-	-	-	-	14	-	-	-	1	6	51	-	-	7	2	1	4	16	14	11	-	-	2	6	5	10	2	-	72	-	38
Corp/Pl decr	-	22	25	-	-	-	2	16	21	8	9	-	3	14	-	2	-	-	19	8	-	-	-	-	-	-	-	-	-	-	-	11	13	-	-	-	-	-	-	4	-	-
Whole Blood	58	130	88	83	73	98	113	177	190	146	92	79	101	141	149	123	60	70	113	167	108	83	83	80	83	122	126	111	106	80	89	186	197	133	81	63	97	98	174	154	97	73
Plasma	83	136	75	78	72	67	115	182	202	155	75	73	101	147	157	120	60	68	104	150	97	80	80	74	85	119	161	104	110	68	73	196	200	141	70	61	108	99	191	206	94	67
Corpuscles	97	84	-	111	79	88	116	155	162	146	94	-	94	125	139	107	65	65	107	120	101	87	82	77	83	127	161	111	93	82	88	176	179	119	72	60	94	98	161	164	104	66
Corp/Pl incr	17	-	-	46	10	-	2	-	-	-	-	-	-	-	-	-	30	-	5	-	2	9	1	2	-	6	-	7	-	20	20	-	-	-	2	-	-	-	-	5	-
Corp/Pl decr	-	38	-	-	-	19	-	15	25	6	2	-	7	15	12	11	-	2	-	8	-	-	-	-	2	-	13	-	16	-	-	-	10	11	16	-	1	13	1	16	21	-	2
Whole Blood	92	81	71	69	40	68	134	241	276	207	82	73	108	153	114	92	78	49	97	190	222	197	74	80	92	140	120	96	92	77	101	132	132	113	119	84	136	219	272	146	63	79
Plasma	92	78	75	60	36	64	156	263	313	224	77	60	100	172	103	82	60	47	94	207	230	202	70	72	87	151	117	103	94	76	91	157	136	107	118	82	136	224	277	141	61	75
Corpuscles	94	81	73	75	46	70	153	186	236	165	74	75	100	139	111	94	80	61	100	187	197	202	87	88	96	117	108	87	96	74	95	-	109	113	115	85	120	184	230	157	77	81
Corp/Pl incr	2	4	-	24	28	9	-	-	-	-	-	25	-	-	8	14	35	30	8	-	-	-	-	24	-	-	-	-	2	-	4	-	-	5	-	3	-	-	-	-	26	8
Corp/Pl decr	-	-	3	-	-	-	2	29	25	26	4	-	-	19	-	-	-	-	-	10	15	-	-	-	1	23	8	16	-	3	-	19	-	3	-	-	12	16	17	3	-	-
Whole Blood	90	190	188	151	68	79	89	146	190	116	73	62	134	200	167	162	107	88	94	143	153	130	68	73	96	174	156	116	81	78	105	133	126	113	117	87	92	171	236	159	86	78
Plasma	90	206	186	146	59	72	92	145	189	113	57	57	125	183	172	162	107	75	83	150	130	131	65	62	99	177	148	106	70	60	102	139	125	111	118	76	92	176	265	166	67	60
Corpuscles	106	169	190	160	91	85	106	139	186	112	70	65	-	187	141	150	106	94	99	141	158	136	74	81	95	152	129	111	-	77	118	101	122	104	105	87	88	128	171	140	82	82
Corp/Pl incr	17	-	2	10	54	15	15	-	-	-	23	10	-	-	-	-	-	25	19	-	6	4	14	20	-	-	-	-4	-	28	16	-	-	-	-	14	-	-	-	10	37
Corp/Pl decr	-	19	-	-	-	-	-	5	18	1	-	-	-	2	18	8	1	-	-	6	-	-	-	-	4	14	10	-	-	-	-	28	2	6	11	-	-	15	18	36	16	-	-
Whole Blood	118	187	150	154	89	116	98	127	145	139	79	63	98	167	173	87	78	77	120	196	158	133	100	81	104	159	193	145	98	76	90	145	152	139	73	84	107	191	256	200	80	74
Plasma	116	196	165	152	89	107	86	124	163	136	76	67	92	178	178	48	76	76	128	207	146	128	107	79	88	162	189	173	65	67	91	153	159	126	61	62	96	230	282	230	66	61
Corpuscles	110	171	132	146	-	113	94	106	129	120	101	70	-	146	187	71	95	76	124	176	158	136	116	102	96	155	162	146	104	75	91	143	122	131	71	58	104	212	234	150	75	73
Corp/Pl incr	-	-	-	-	-	26	10	-	-	-	32	4	-	-	-	49	25	-	-	-	8	4	4	28	10	2	-	-	21	12	-	-	-	.4	16	-	8	-	-	14	20
Corp/Pl decr	5	13	15	4	-	-	-	14	9	12	-	-	-	18	23	-	-	-	3	15	-	-	-	-	-	-	15	16	-	-	-	7	25	-	-	7	-	8	19	38	-	-
Whole Blood	126	182	194	116	108	92	96	126	132	88	78	64	89	116	87	105	63	70	111	150	145	141	-	85	98	117	80	86	76	78	96	139	86	142	120	68	133	173	207	154	67	80
Plasma	126	185	194	114	90	91	91	179	141	93	75	62	92	98	81	98	82	69	110	150	156	136	106	64	71	108	75	63	65	55	97	146	75	-	110	58	136	184	226	147	82	73
Corpuscles	119	170	187	115	106	87	94	130	117	90	73	-	105	100	-	102	70	68	114	150	141	206	133	-	88	112	85	100	76	87	95	124	75	141	123	67	106	153	187	92	84	46
Corp/Pl incr	-	-	-	-	18	-	3	-	-	-	-	-	14	2	-	4	-	-	4	-	-	48	35	-	-	-	-	-	-	-	-	-	-	12	15	-	-	-	-	2	-
Corp/Pl decr	6	8	4	1	-	5	-	28	17	3	3	-	-	-	-	15	1	-	-	-	10	-	-	-	-	-	-	-	-	-	2	15	-	-	-	-	22	28	18	38	-	37
Whole Blood	123	173	191	176	121	101	108	138	151	105	70	82	104	153	142	100	72	78	94	158	122	136	120	82	68	121	130	114	94	53	99	208	197	98	77	85	116	214	179	114	73	89
Plasma	123	167	183	176	122	90	100	143	185	95	62	80	160	164	151	98	67	65	98	158	121	147	111	82	77	121	123	96	87	45	103	245	197	94	82	63	112	224	189	111	63	82
Corpuscles	118	181	171	104	123	103	-	-	-	120	81	-	136	143	122	92	68	75	94	156	122	129	118	88	90	117	120	108	107	53	104	192	178	99	84	83	104	169	128	111	69	93
Corp/Pl incr	-	-	-	-	-	14	-	-	-	26	30	-	-	-	-	1	19	-	-	-	-	-	6	7	16	-	-	12	23	18	1	-	-	5	2	-	-	-	-	-	10	14
Corp/Pl decr	4	10	7	42	-	-	-	-	-	-	-	-	18	14	19	-	-	-	4	8	-	13	-	-	-	4	2	-	-	-	-	21	9	-	-	-	7	28	33	-	-	-
Whole Blood	98	146	121	89	86	67	102	170	122	126	79	-	100	171	161	104	73	67	102	158	121	94	89	85	113	130	131	177	111	90	78	145	107	104	85	78	118	187	180	164	89	116
Plasma	99	182	125	88	83	68	94	184	116	110	64	-	84	185	163	104	70	59	127	170	102	77	81	90	108	139	139	179	94	78	76	135	106	106	91	68	116	196	155	152	89	107
Corpuscles	100	123	125	98	85	69	103	169	125	110	98	-	-	143	121	100	75	68	147	167	150	121	89	-	105	132	126	172	109	83	73	119	99	97	-	76	110	171	132	146	-	113
Corp/Pl incr	1	-	-	8	2	2	10	-	-	8	-	53	-	-	-	-	7	15	16	-	49	84	5	-	-	-	-	-	16	6	-	-	-	-	-	12	51	-	-	-	-	8
Corp/Pl decr	-	19	2	-	-	-	-	-	8	-	-	-	-	22	26	4	-	-	-	2	-	-	-	-	-	5	9	4	-	-	4	10	7	9	-	-	-	13	18	4	-	-
Whole Blood	87	202	93	87	60	61	112	121	95	84	72	73	142	166	117	135	120	63	184	219	260	182	114	91	103	165	121	122	94	73	88	142	143	112	74	81	145	254	261	117	87	110
Plasma	82	223	94	84	62	58	84	121	88	72	61	62	128	171	117	121	114	70	148	219	286	176	83	72	94	166	129	130	83	71	89	141	120	113	56	73	185	285	285	113	82	-
Corpuscles	80	183	84	74	63	73	109	103	89	-	67	62	-	161	110	126	116	90	125	187	221	155	98	77	98	151	123	128	102	94	108	136	-	112	74	74	140	212	197	105	76	-
Corp/Pl incr	-	-	-	-	-3	26	30	-	1	-	9	-	-	-	-	-	4	2	-	-	-	-	17	2	4	-	-	-	23	32	21	-	-	-	-	-	-	-	-	-	-	-
Corp/Pl decr	2	31	10	12	-	-	-	15	-	-	-	-	-	6	6	-	-	-	14	15	22	12	-	-	-	4	5	2	-	-	-	4	-	1	-	-	10	26	31	7	8	-
Whole Blood	96	183	98	92	77	86	128	192	263	208	113	73	106	109	98	82	94	88	94	150	158	136	68	84	104	160	161	177	101	60	165	226	303	190	111	86						
Plasma	82	157	87	74	68	83	141	201	260	241	111	70	95	108	108	73	79	71	94	161	194	145	75	85	95	157	171	192	104	63	147	252	333	212	114	74						
Corpuscles	97	160	97	107	85	-	111	-	230	185	104	90	92	74	100	89	84	92	94	145	158	146	146	95	106	136	167	178	92	76	159	208	261	161	96	84						
Corp/Pl incr	16	-	12	44	25	-	-	-	-	-	-	29	-	-	-	21	6	28	-	-	-	94	12	11	-	-	-	77	8	-	-	-	14								
Corp/Pl decr	-	5	-	-	-	-	21	-	18	24	7	-	4	32	8	-	-	-	-	11	19	-	-	-	-	1	3	8	12	-	-	18	22	24	14	-						

TABLE II

RELATION OF PLASMA SUGAR TO CORPUSCLE SUGAR IN DIABETIC PATIENTS
(Estimations made during glucose tolerance tests)

Hours after ingestion of glucose

	0	½	1	2	3	4	0	½	1	2	3	4	0	½	1	2	3	4	0	½	1	2	3	4	0	½	1	2	3	4	0	½	1	2	3	4
Whole Blood	104	200	269	258	200	136	163	284	309	348	336	267	162	292	309	400	400	333	234	294	326	366	323	303	193	252	366	416	292	226	202	294	400	497	485	380	122	216	240	333	282	200	184	306	344	440	423	333
Plasma	108	193	261	261	261	155	187	288	344	384	344	267	167	293	333	423	440	370	236	350	333	375	330	315	193	252	366	416	292	226	200	306	410	516	506	416	118	218	230	333	306	207	201	333	380	497	460	353
Corpuscles	199	167	258	234	191	123	156	204	252	319	280	258	141	238	288	333	353	250	214	-	316	333	316	248	196	236	306	348	234	224	201	275	366	447	434	340	132	191	-	282	250	207	155	272	309	380	367	277
Corp/Pl incr	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	12	-	-	-	-	-	-	-	-	-	-	-
Corp/Pl decr	9	14	1	10	27	9	17	30	27	17	19	3	16	18	13	21	20	33	10	-	5	12	4	21	7	11	15	19	18	5	-	10	10	13	15	19	-	12	-	16	19	-	23	19	19	23	23	22
Whole Blood	159	219	275	294	182	150	160	234	275	357	282	234	103	196	336	525	485	389	101	177	224	226	171	155	214	258	270	387	428	319	142	210	278	243	192	124	102	184	224	241	161	217	145	200	263	333	267	168
Plasma	160	227	294	-	178	117	180	238	303	405	316	241	95	206	375	552	525	400	103	115	252	226	172	162	221	267	280	366	434	428	150	208	300	256	193	124	72	191	226	241	141	32	147	201	292	366	280	174
Corpuscles	140	189	227	-	176	129	141	161	208	300	254	157	84	-	208	484	389	309	100	164	216	207	155	140	206	243	261	344	416	370	142	185	226	243	177	120	96	168	189	209	143	-	133	138	226	292	221	139
Corp/Pl incr	-	-	-	-	-	10	-	-	-	-	-	-	-	42	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	5	-	-	-	1	-	-	-	-	-	-	-
Corp/Pl decr	12	16	23	-	1	-	22	33	32	26	20	35	12	-	44	18	26	23	3	-	15	9	10	14	7	9	7	6	4	14	5	11	25	5	8	3	-	12	17	14	-	-	10	31	23	21	21	20
Whole Blood	162	267	313	333	282	212	176	241	252	340	246	133	405	497	600	626	563	490	136	143	182	246	270	275	286	294	326	447	389	309	306	380	394	506	485	450	148	272	212	232	183	86	142	256	234	191	124	106
Plasma	171	300	344	400	319	217	187	254	261	366	254	138	440	543	626	680	600	506	130	156	174	265	294	288	270	323	-	475	428	333	319	410	416	552	490	440	153	288	234	234	182	87	152	267	248	190	115	99
Corpuscles	164	236	275	309	267	190	155	173	227	254	224	117	319	410	553	563	576	-	124	150	145	230	248	252	243	288	306	394	316	265	275	323	323	434	400	349	136	256	170	200	-	67	-	219	196	177	111	105
Corp/Pl incr	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
Corp/Pl decr	4	21	20	23	17	12	18	32	13	31	12	15	28	25	43	17	15	-	5	5	17	13	15	13	10	11	-	17	26	21	13	21	23	22	19	22	11	18	28	15	-	23	-	18	21	7	4	-
Whole Blood	214	333	384	416	303	250	90	207	219	200	180	70	132	221	282	254	182	121	106	189	222	272	300	303	124	147	228	212	132	96	187	300	400	460	386	280	300	400	482	530	442	342	107	202	204	228	150	78
Plasma	261	394	434	460	375	270	72	250	207	204	171	65	131	200	306	270	192	119	99	186	232	300	299	303	122	148	238	204	125	88	206	348	454	543	357	306	306	400	482	544	442	374	107	199	224	252	142	75
Corpuscles	197	286	375	326	275	206	74	180	197	182	138	60	122	197	263	234	130	71	105	167	200	261	272	267	121	139	194	193	116	88	161	285	349	389	272	219	-	360	374	428	366	280	111	184	190	207	141	80
Corp/Pl incr	-	-	-	-	-	-	2	-	-	-	-	-	6	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	4	-	-	-	-	-
Corp/Pl decr	25	25	14	29	27	24	-	28	5	11	20	8	8	2	14	14	33	40	-	10	14	13	9	12	-	6	19	7	7	-	22	18	24	28	24	29	-	10	22	21	17	25	-	7	15	12	1	-	7
Whole Blood	111	194	288	309	206	123	214	227	200	118	100	75	143	130	150	98	143	109	98	184	196	300	309	300	129	190	323	384	447	353	121	185	217	236	165	87	147	212	252	306	227	126	135	252	330	428	375	254
Plasma	118	217	300	344	222	122	224	241	204	116	100	67	130	127	153	146	132	99	91	168	206	294	300	313	132	197	340	428	467	375	122	190	250	243	164	78	154	258	272	350	241	135	156	297	344	490	380	303
Corpuscles	117	161	204	272	187	106	179	206	178	106	100	46	130	128	147	150	128	111	94	146	-	309	294	254	119	167	267	336	394	306	98	152	190	221	164	87	140	212	219	258	204	122	133	234	310	370	309	200
Corp/Pl incr	1	-	-	-	-	-	-	-	-	-	-	-	-	-	-	3	-	12	3	-	-	5	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
Corp/Pl decr	-	26	32	21	16	13	24	14	13	9	-	31	-	-	4	-	3	-	-	13	-	-	2	19	10	15	22	21	16	18	20	20	24	10	3	-	9	18	20	22	15	8	15	21	10	24	19	34
Whole Blood	252	275	366	467	476	348	143	250	316	394	263	197	221	297	210	178	161	177	131	176	200	230	200	101	138	230	275	389	353	280	142	240	319	366	389	297	250	292	277	454	375	294	118	196	288	149	135	128
Plasma	270	294	428	506	516	405	145	258	362	428	292	201	270	-	212	219	192	162	130	176	200	240	214	100	140	246	292	416	389	272	199	238	362	389	405	309	246	319	319	506	400	309	101	199	297	130	141	125
Corpuscles	204	222	323	-	384	297	-	227	263	313	243	174	202	280	187	171	142	155	121	158	164	200	176	100	132	222	272	348	294	234	104	207	280	333	348	252	213	243	238	440	333	272	119	177	240	143	142	133
Corp/Pl incr	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	16	-	-	-	-	-
Corp/Pl decr	25	25	25	-	26	27	-	10	28	27	17	14	25	-	12	22	22	15	7	10	18	18	17	-	6	10	7	16	25	14	19	14	23	15	14	19	13	24	25	13	17	12	-	11	40	-	-	6
Whole Blood	167	292	394	400	306	248	131	200	261	238	171	107	134	194	226	173	130	64	136	216	300	400	460	314	263	344	424	423	384	323	126	287	344	516	552	416	129	172	222	238	189	96	213	275	330	485	394	335
Plasma	174	330	434	454	344	261	132	207	265	254	173	102	141	206	241	176	140	54	126	230	243	429	426	352	280	384	410	475	360	313	117	267	362	525	567	485	122	169	248	243	199	95	219	282	330	506	447	333
Corpuscles	141	265	344	330	272	197	142	171	243	222	153	103	97	132	154	147	122	-	99	180	254	348	352	302	246	309	326	390	333	272	116	241	241	366	440	389	122	166	193	221	173	90	180	226	267	384	270	267
Corp/Pl incr	-	-	-	-	-	-	7	-	-	-	-	-	-	-	-	-	-	-	-	-	-	4	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	2	23	9	13	6	-	-	-	-	-	-
Corp/Pl decr	19	20	21	28	21	25	-	18	8	12	12	-	31	36	36	16	13	-	21	22	-	19	18	15	12	20	20	18	12	15	1	10	34	30	25	20	-	2	23	9	13	6	18	20	19	24	17	20
Whole Blood	187	300	333	460	497	400	113	206	199	199	178	139	173	240	243	224	164	126	167	250	236	187	180	145	258	356	406	534	412	-	112	143	187	193	149	79	204	280	348	447	410	344	130	234	306	344	217	155
Plasma	187	300	375	484	497	400	98	204	224	199	192	145	168	243	272	214	150	108	177	288	292	199	190	142	252	352	428	508	374	282	105	158	190	199	145	67	210	306	405	506	464	384	130	230	319	375	346	316
Corpuscles	147	272	272	375	460	375	103	201	194	194	177	159	138	217	194	191	125	-	161	236	240	170	161	139	252	312	400	500	368	-	118	150	179	210	160	79	184	258	316	416	375	309	111	175	238	292	213	152
Corp/Pl incr	4	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	18	-	-	-	-	-	-	-	-	-	12	-	-	5	10	17	-	-	-	-	-	-	-	-	-	-	-	-	-
Corp/Pl decr	22	10	28	18	8	7	-	2	14	11	18	9	18	18	29	11	17	-	9	18	-	15	16	2	-	11	6	1	2	-	-	5	6	-	-	-	13	15	22	18	17	20	15	24	25	22	13	2
Whole Blood	250	333	375	497	506	497	109	178	213	185	136	95	104	158	248	174	137	136	268	428	442	500	428	334	286	352	444	500	560	588	130	197	272	313	163	142	256	370	405	394	303	258	133	219	236	228	134	90
Plasma	250	353	353	497	543	497	107	186	219	154	133	88	108	194	258	169	129	117	264	460	460	522	428	342	272	352	438	444	544	584	120	227	272	319	178	130	277	389	428	428	323	280	116	210	234	236	124	89
Corpuscles	230	300	333	476	497	467	110	171	207	152	131	92	103	184	256	160	114	128	214	360	420	428	352	272	267	-	326	400	480	482	117	178	230	282	219	133	236	333	344	428	267	267	126	216	215	196	124	-
Corp/Pl incr	-	-	-	-	-	-	2	-	-	-	-	4	-	-	-	-	-	9	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	8	-	-	-	-
Corp/Pl decr	9	15	6	5	9	6	-	8	6	2	2	-	5	6	1	6	12	-	19	22	26	18	18	21	2	-	25	20	12	18	2	2	22	15	12	-	-	15	14	20	-	18	5	-	14	9	18	-	-
Whole Blood	105	173	250	187	160	63	206	261	313	357	467	323	114	217	275	192	124	91	268	334	408	400	214	145	230	374	428	478	438	324	100	176	164	167	146	107	110	178	184	161	135	116	99	224	201	166	166	84
Plasma	95	187	261	200	136	67	210	319	357	390	454	336	111	230	282	200	129	80	250	334	460	438	214	136	234	382	460	500	404	-	98	184	169	162	142	107	108	187	171	180	131	117	95	238	208	171	185	73
Corpuscles	103	168	230	170	130	67	164	241	256	292	378	252	116	212	258	173	118	-	200	294	400	-	-	234	200	306	342	384	428	325	107	-	164	161	145	115	116	-	177	134	134	113	88	208	200	-	183	81
Corp/Pl incr	8	-	-	-	-	-	-	-	-	-	-	-	4	-	-	-	-	-	-	-	-	-	-	72	-	-	-	-	-	-	9	-	-	2	7	7	-	-	-	-	-	-	-	-	-	-	-	11
Corp/Pl decr	-	10	20	15	5	-	12	24	28	23	18	31	-	8	12	14	9	-	20	12	13	-	-	-	15	20	26	23	-	6	-	-	2	1	-	-	-	8	25	4	4	8	13	4	-	1	-	-	-
Whole Blood	125	181	219	228	196	98	107	138	172	200	204	169	384	430	543	666	563	506	120	214	350	297	297	171	153	227	300	246	165	125	145	186	226	222	158	130	192	292	340	466	326	267	336	467	467	535	380	348
Plasma	117	161	246	228	189	98	109	171	185	201	206	174	405	516	587	680	587	543	117	230	280	316	297	176	156	238	240	250	165	121	146	185	254	171	161	116	194	309	344	423	344	277	389	516	516	612	454	342
Corpuscles	104	136	199	213	194	98	96	128	142	179	171	158	333	423	-	575	525	460	-	200	230	-	206	-	150	202	272	252	182	122	143	171	217	162	164	130	170	256	294	348	294	254	303	362	370	490	344	-
Corp/Pl incr	-	-	-	-	-	2	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	13	1	-	-	-	-	-	14	12	-	-	-	-	-	-	-	-	-	-	-	-
Corp/Pl decr	12	15	19	7	-	-	12	25	23	11	18	9	19	18	-	15	10	15	-	13	8	-	30	-	4	15	-	-	8	-	2	8	15	5	-	-	13	17	15	23	15	8	22	30	28	20	24	-
Whole Blood	206	294	319	467	423	348	92	125	140	152	147	57	208	288	330	384	344	303	154	290	334	366	250	187	154	239	326	375	353	294	142	214	272	200	125	98	152	213	248	323	348	316	109	256	282	265	191	180
Plasma	228	330	362	497	475	389	84	128	141	155	155	42	221	303	326	400	380	333	155	286	374	368	306	210	153	250	333	389	375	306	133	226	280	202	116	91	184	224	261	366	366	326	97	256	306	288	191	107
Corpuscles	176	280	360	394	357	340	90	108	140	131	196	95	191	263	265	355	323	275	143	219	268	310	240	176	153	220	277	353	393	-	131	193	250	192	125	100	136	200	230	290	-	258	113	246	277	236	170	180
Corp/Pl incr	-	-	-	-	-	-	7	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	1	-	-	-	8	10	-	-	-	-	-	-	16	-	-	-	-	-
Corp/Pl decr	23	15	31	21	25	13	-	16	1	16	6	-	14	13	19	12	15	18	28	24	28	16	22	16	-	12	17	10	14	-	1	15	11	5	-	-	12	11	12	21	-	21	-	14	10	18	11	-	12
Whole Blood	139	189	236	285	255	206	333	366	485	583	506	460	157	267	141	243	188	117	182	316	339	340	243	187	267	366	428	575	563	440	123	179	221	405	428	366	173	300	389	447	418	362						
Plasma	133	202	246	300	252	219	340	400	525	600	543	497	161	280	204	187	171	129	192	346	352	380	236	184	282	375	425	563	575	460	124	207	227	416	454	375	206	344	416	485	428	326						
Corpuscles	131	167	191	285	-	204	254	326	389	467	467	389	-	155	142	187	146	111	148	294	294	261	186	173	234	297	333	506	497	384	116	-	192	362	416	348	169	261	375	394	416	340						
Corp/Pl incr	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-						
Corp/Pl decr	2	17	22	5	-	7	26	19	26	22	14	22	-	25	21	-	15	14	18	30	17	7	22	7	17	21	20	10	14	17	6	-	15	13	9	8	18	25	10	19	3	6						

was 321 per cent, while among the diabetic subjects it was only 185 per cent. This test, therefore, confirmed our previous observation that in diabetes the corpuscles are less permeable to sugar than they are normally. Whether in diabetes the corpuscles have more nearly reached their saturation limit cannot be stated, but one would hardly suspect that to be the case, as a very high sugar content of the corpuscles may be observed in cases of diabetes, and this would indicate a tremendous capacity for taking in sugar.

TABLE III

A COMPARISON OF THE CORPUSCULAR SUGAR WITH THE PLASMA SUGAR IN NONDIABETIC AND DIABETIC CASES

	NONDIABETIC	DIABETIC
Total number of examinations	497	572
Total per centile increase	4212	438
Total number of examinations	246	44
Average per centile increase	17.12	9.95
Total per centile decrease	2879	8084
Total number of examinations	236	511
Average per centile decrease	12.20	15.82
Number of cases in which corpuscle sugar equalled plasma sugar	20	17

TABLE IV

PERMEABILITY OF RED BLOOD CELLS TO SUGAR

CORPUSCLES EXPOSED TO 1000 MG. OF GLUCOSE PER 100 C.C. OF N/10 CaCl SOLUTION

	NONDIABETIC	DIABETIC
Total number of cases	19	23
Total per centile increase of corpuscular sugar	6100	4261
Average per centile increase of corpuscular sugar	321	185

That corpuscles should not be centrifuged and then washed by normal saline solution before their sugar content is estimated, as has been done by some authors, can be clearly seen from Table V. When the corpuscles are washed part of the sugar is washed out of them, for osmosis does not cease, just as when corpuscles are suspended in a hypertonic sugar solution, sugar is introduced into them. Therefore, the washing of corpuscles before their sugar content is estimated invalidates the results of the test, and data compiled from such estimations must be disregarded.

TABLE V

THE LOSS OF SUGAR FROM THE CORPUSCLES AS THE RESULT OF WASHING THEM WITH NORMAL SALINE SOLUTION

			PER CENT	PER CENT LOSS
Case I.	Corp. sugar before washing	204	100	
	Corp. sugar after 15 minutes	94	46	54
	Corp. sugar after 30 minutes	23	11	87
Case II.	Corp. sugar before washing	170	100	
	Corp. sugar after 15 minutes	78	46	54
	Corp. sugar after 30 minutes	38	22	78

CONCLUSIONS

1. The sugar content of the red blood corpuscles in the diabetic individual is strikingly lower than the sugar content of the surrounding plasma.

2. As the amount of the sugar in the blood of the diabetic individual increases, the increase occurs first in the plasma and does not appear until later in the red corpuscles. As the level of the plasma sugar falls, the corpuscular sugar as a rule keeps pace with it. Thus one rarely finds a higher sugar level in the corpuscles than in the plasma at the end of a glucose tolerance test, when the blood-sugar curve is descending.

3. This relationship is not altered in severe cases of diabetes, that is, in cases in which the blood sugar is very high.

4. In general, in the nondiabetic individual the corpuscles have a lower sugar content than the plasma, but this is not so marked as in the diabetic subject.

5. In the nondiabetic subject, when the sugar in the plasma increases, the corpuscular sugar rises more than it does in the case of a diabetic; but when the sugar curve is descending the corpuscles do not release their sugar so freely, and thus, as a general rule, one finds a higher level in the corpuscular sugar than in the plasma sugar at the end of the curve.

6. A study of 1,069 blood-sugar estimations shows that in nondiabetic individuals the ratio of the average increase of the sugar in the corpuscles, as compared with that in the plasma, to the average decrease was 17.12 to 12.20, while in diabetic individuals the corresponding ratio was 9.95 to 15.82.

7. By suspending red corpuscles in 1,000 mg. per 100 c.c. of glucose in a N/10 NaCl solution, the average increase of the corpuscular sugar after two hours was found to be 321 per cent in corpuscles from nondiabetic individuals, and 185 per cent in those from diabetic individuals.

8. The washing of corpuscles in normal saline solution before their sugar content is estimated is contraindicated, as the sugar is washed out of the corpuscles and the estimations are therefore of no value.

9. The present study would suggest that in diabetes the corpuscles have a lesser capacity to take in and to hold sugar than normally. Whether it is the decreased amount of insulin present or some other condition which is the dominant factor, future studies should disclose.

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EXPERIMENTAL STUDIES IN DEPROTEINIZING BLOOD SERUM

Preliminary Report

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THE chemical method of precipitating protein from solution by the use of the salts of heavy metals, and also by the use of "alkaloidal reagents," is a familiar study in biochemistry.

This experimental method employs heavy metals, not as a salt, but in their pure state, reacting through the application of an electric current.

For experimental purposes, a cell is prepared by fusing small gauge platinum wires through the wall of a large-size test tube. The fusing makes a "water tight" joint, and leaves one end of each wire protruding into the lumen of the tube. Upon the ends of the wires inside the tube are hung metal plates which constitute the electrodes. To the ends of the wires outside the tube are attached the lead wires from the generator. Other metals may produce the same physiochemical reaction, but the only one I have so far experimented with successfully has been pure gold.

When blood serum in its pure state or diluted with physiologic or hypertonic salt solution is placed in the cell, and a direct electric current applied, the protein (both primary and secondary) gradually accumulates upon the anode. If the serum is undiluted or slightly diluted, the protein will remain adherent to the gold anode. When the protein is all precipitated, the current is shut off, and the anode lifted from the cell with the protein adherent to it. If the serum is highly diluted and fresh, the protein will shed from the gold anode and settle to the bottom of the cell so that the protein-free solution can be pipetted off from above and between the electrodes. Platinum makes an excellent electrode, but will not cause the precipitation of protein when used as the anode, consequently the use of a platinum plate as the cathode and a gold plate as the anode makes a suitable cell.

Fig. 1 is a photostat of one of the cells used. It is a glass tube, open at the top. The short plate in the picture is the platinum cathode and the larger plate is the gold anode. The amount of serum or serum solution placed in the cell should just cover the electrodes. The current may be furnished by any machine that will produce a direct current. The amount of current has been varied between 100 volts with 30 milliamperes and 20,000 volts with 8 milliamperes, with the same deproteinizing action.

The remaining protein-free solution is slightly alkaline. The degree of alkalinity will depend upon the amount of protein that has been precipitated and the hypertonicity of the solution. The alkalinity of the electrolyte can be held at the original P_H of the antitoxin by incasing the platinum cathode in a dializer and keeping the plate bathed in neutral physiologic salt solution. If the serum is undiluted or slightly diluted, it is best to make the solution hypertonic to insure and hasten the complete precipitation of the protein.

To determine if all the protein has been precipitated, a small portion of the solution can be pipetted from between the electrodes and tested. If the serum is highly diluted, the solution may be boiled as pipetted from the cell, and if the protein is removed, the solution remains perfectly clear after boiling. If the serum is concentrated, as in the case of diphtheria antitoxin, there will remain in solution so much antitoxic substance, which is itself precipitated by heat, that the boiling of the solution as pipetted from the cell will not signify the complete disappearance of the protein. In this case, a preliminary step is necessary before boiling. As the solution is alkaline after the application of the current, the antitoxic element of the serum will remain in alkaline solution, but is immediately precipitated when the solution is made

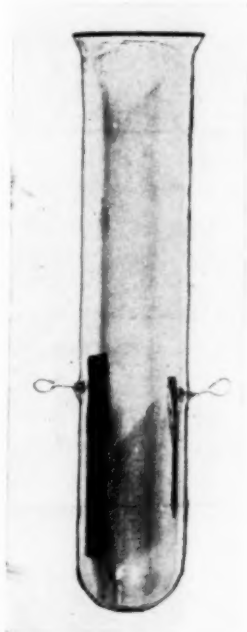

Fig. 1.—Cell for electrically precipitating serum protein.

very slightly acid. Consequently, it is necessary to render the portion removed for protein test very slightly acid and filter before boiling.

The purpose of this method of deproteinizing serum is to precipitate the protein substance and remove it from the antibodies or antitoxic elements. This leaves these elements in a slightly alkaline salt solution. The antitoxic elements keep better, act quicker and with greater precision after the protein is removed. This improves the application of the complement-fixation test, as well as antitoxins for therapeutic use.

Hemolytic serum or amboceptor, as used in the Wassermann test, is simply the blood serum of a rabbit that has been immunized to the red blood cells of, usually, the human being or sheep. I have deproteinized antisheep serum, after which it acts more precisely than before deproteinization. A portion of the deproteinized serum, diluted to titer, was left at room temperature

for twenty days. At the end of that time, none of its hemolytic property had been lost.

The syphilitic antibodies remain after deproteinizing the serum of a syphilitic patient. A positive Wassermann is obtained just the same after removing the protein as before. The only difference is that the test on the deproteinized serum behaves more precisely, and positive results are obtained with less serum. If the patient does not have syphilis, the test is clearly negative.

Gonococcus fixation tests are clearer.

It seems that in tuberculosis the elements separated are both toxin and antitoxin. If the patient has active tuberculosis with temperature there are no antibodies present, but tuberculin is present. If the patient does not have active tuberculosis with increased temperature there is a certain measure of tuberculous antibodies present. This is indicated by the fact that tuberculin will increase the rate of hemolysis in the hemolytic phase of the complement-fixation test. This is shown by running complement-fixation tests upon the deproteinized serum of tuberculous and nontuberculous patients, using a carefully standardized old tuberculin as antigen. (The antigen should also be deproteinized after dilution.) I have made twenty of these tests from patients at Dr. R. B. Homan's Sanatorium, who were selected by him. The sera were each given a number and sent to my laboratory, without indicating which were tuberculous and which were not tuberculous. The very sick patients were quickly recognized by the rapid hemolysis. All who were running any degree of increased temperature from tuberculous infection were separated from the others.

This is very interesting, because the deproteinizing of the serum and antigen makes the separation possible; and because those showing the positive complement-fixation tests are the nontuberculous patients. Those that show the most rapidly negative complement-fixation tests are the sickest from tuberculosis.

Deproteinized guinea pig serum leaves the complement in protein-free solution. This keeps longer and acts better.

Antitoxic sera for therapeutic use are materially improved. The removal of the protein takes away the element that produces anaphylactic shock, with the violent skin reactions that too frequently follow their use. The protein-free antibody solution is not irritating or toxic. Its physiologic action is quicker. It is possible to precipitate out the antitoxic element by rendering the alkaline solution slightly acid. The precipitate can then be dissolved in a minimum amount of very slightly alkalinized physiologic salt solution.

CONCLUSIONS

1. The deproteinizing of blood serum by electrolysis is practical and not difficult.

2. It separates and removes the total protein from the toxic or antitoxic elements of the serum.

3. The antitoxic elements act in their specific manner more precisely after the protein is removed.

4. The antitoxic element with the protein removed keeps better and tolerates a higher temperature.

5. The removal of the protein from the serum by electrolysis makes the complement-fixation test more reliable.

6. It makes the laboratory detection of tuberculosis possible from the blood serum.

7. It removes the objectionable, and at times dangerous, element from antitoxic sera.

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STUDIES ON LACTIC ACID IN THE BLOOD*

I. The Effect of Glucose and Insulin

By Harry Koster, M.D., F.A.C.S., M. Goldzieher, M.D., W. S. Collens, M.D., and I. E. Gerber, M.D., Brooklyn, N. Y.

IN A RECENT paper we[1] demonstrated that in the postoperative state there is no evidence of the utilization of glucose, intravenously administered, using the respiratory quotient as an indicator. Since this glucose disappeared from the blood stream without any evidence of its oxidation, the question concerning its fate arose. Among other possibilities we considered its conversion into lactic acid. A review of the literature discloses the following facts:

Collazo and Lewicki[2] showed a rise in lactic acid after administration of glucose. Katayama and Killian[3] confirmed their results. The latter also obtained a rise following the administration of glucose and insulin. Briggs and his coworkers[4] showed a rise in lactic acid with a drop in the blood sugar by the use of insulin alone. Collazo and Supniewski[5] confirmed these last observations. Tolstoi, et al.[6] obtained similar results inconstantly.

Contradictory results were obtained by other observers. Isaac and Adler[7] found no change in lactic acid following insulin and glucose. Mendel, Engel and Goldscheider[8] were unable to obtain a rise in lactic acid following glucose or insulin. Best and Ridout[9] did not find a rise following insulin injections. C. F. Cori,[10] Best and Scott,[11] and Servantie,[12] working independently, arrived at the same conclusion. Otto Jervell[13] found no rise following carbohydrate feeding.

These conflicting results led us to make the following studies:

The effect of intravenously injected (a) glucose, (b) insulin, and (c) glucose and insulin on the lactic acid level in the blood.

METHOD

These experiments were all performed on nondiabetic adult patients. The lactic acid determinations were done according to the method of Friedemann,

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Cotonio and Shaffer.[14] The blood was drawn without stasis, using sodium oxalate to prevent clotting. The proteins were precipitated according to the method of Folin and Wu, and the filtrate was divided into two portions. On one portion quantitative blood-sugar determinations were made. The other portion was treated with 10 per cent $CuSO_4$ and 5 per cent $Ca(OH)_2$ to remove sugar and other interfering substances, such as creatinin and uric acid. This desugarized filtrate was then used for the determination of lactic acid. Two determinations were made on each blood specimen and were found to check within the range of 5 per cent error for the method. Our results, in each case, represent the average of two determinations.

The insulin and glucose were given intravenously. The amount of glucose used was 250 c.c. of a 20 per cent solution.

RESULTS

Group I. Effect of glucose.—In this series as shown in Table I, after the intravenous administration of glucose and the determinations of lactic acid made at hourly intervals for four hours, there was no rise found in the blood lactic acid level. In several instances there was a slight decrease.

TABLE I

THE EFFECT OF INTRAVENOUS GLUCOSE

	W. W.		B. N.		M. G.		S. S.		H. H.	
	SUGAR	LACTIC ACID	SUGAR	LACTIC ACID	SUGAR	LACTIC ACID	SUGAR	LACTIC ACID	SUGAR	LACTIC ACID
	MG.	100 C.C.	MG.	100 C.C.	MG.	100 C.C.	MG.	100 C.C.	MG.	100 C.C.
Fasting	80	33.08	141	42.35	91	20.14	122	16.14*	93	23.29
Glucose	50 grams		50 grams		50 grams		50 grams		50 grams	
1 hour	129	17.44	156	22.05	100	17.10	148	21.59	136	19.80
2 hours	65	21.60	113	25.43	69	8.55	163	15.72	115	12.26
3 hours	77	22.95	60	19.74			141	15.22	79	16.65
4 hours	71	21.60	73	28.67	98	15.44	127	15.45	73	13.50

*Following the intravenous glucose this patient had a mild chill lasting about twenty minutes. This is the only patient in whom we obtained a rise in lactic acid immediately after glucose.

Group II. Effect of insulin.—With insulin alone, determinations made at half-hourly intervals for a period of two hours showed no significant changes in the blood lactic acid content, the variations being within normal limits.

TABLE II

THE EFFECT OF INTRAVENOUS INSULIN

	R. P.		M. S.		S. F.	
	SUGAR	LACTIC ACID	SUGAR	LACTIC ACID	SUGAR	LACTIC ACID
	MG.	100 C.C.	MG.	100 C.C.	MG.	100 C.C.
Fasting	82	13.50	87	15.75	92	22.68
Insulin	12 units		12 units		15 units	
15 min.	62	16.65				
30 min.	38*	14.40	47*	16.87	31*	24.53
60 min.	61	11.68	54	17.55	39	29.70
90 min.	73	10.35	83	17.37	43	31.72
120 min.	81	14.18	85	12.26	65	24.30

*Hypoglycemic reaction. Sweating, weakness, headache, dizziness, and restlessness. No convulsions or tremors.

Group III. Effect of glucose and insulin.—In this series, where insulin was given one half hour after intravenous glucose injection and determinations were made at hourly intervals for a period of three hours, there were also no significant changes in the blood lactic acid level.

TABLE III

THE EFFECT OF INTRAVENOUS GLUCOSE AND INSULIN

	H. B.		N. M.	
	SUGAR	LACTIC ACID	SUGAR	LACTIC ACID
	MG.	100 C.C.	MG.	100 C.C.
Fasting	93	12.94	79	14.40
Glucose	40 grams		50 grams	
30 min.	544	12.15	158	16.65
Insulin	12 units		15 units	
30 min.	56	9.12	34	12.60
1 hour	65*	12.38	50*	15.75
2 hours	80	14.63	60	12.66
3 hours	82	11.36	75	15.98

*Hypoglycemic reaction, characterized by sweating, weakness, dizziness, headache, pallor, and restlessness. Patients complained of thirst and dryness of the throat. No convulsions or tremors.

Group IV. Effect of glucose and simultaneous respiratory quotient determinations.—Following the administration of glucose and the determinations of lactic acid one hour later, there was no rise in the lactic acid blood level. Concomitant respiratory quotient determinations showed the characteristic rise.

TABLE IV

THE EFFECT OF INTRAVENOUS GLUCOSE WITH SIMULTANEOUS R/Q DETERMINATIONS

	FASTING			TIME AFTER GLUCOSE	AFTER GLUCOSE		
	SUGAR	LACTIC ACID	R/Q		SUGAR	LACTIC ACID	R/Q
	MG.	100 C.C.			MG.	100 C.C.	
P. K.	106	20.48	0.81	50 min.	239	19.46	1.00
E. M.	92	18.79	0.68	60 min.	216	22.21	0.80
D. G.	88	15.87	0.70	40 min.	238	19.57	0.93
A. S.	95	29.25	0.74	45 min.	184	27.80	0.86

CONCLUSIONS

There is no rise in the lactic acid level after the intravenous administration of glucose, insulin, or both. During the period of these observations glucose was oxidized as shown by the rise in the respiratory quotient. It would seem that lactic acid is not an intermediary stage in the metabolism of intravenously administered glucose.

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THE EFFECT OF ALLERGIC REACTIONS ON THE COURSE OF NONALLERGIC DISEASES*

By Warren T. Vaughan, M.D., Richmond, Va.

THE victim of bronchial asthma, hay fever, or urticaria, consulting a physician, presents an acute, sometimes almost tragic, situation which requires immediate relief. His only interest at the time is the one acute condition and the doctor's immediate aim is the relief of the active emergency. His next aim is the finding and removal of the exciting causes. Fortunately, since the development of the concept of protein sensitization as a cause for these allergic diseases, relief, entire or partial, is obtained in a large number of instances. As a consequence, the man who has developed a special interest and proficiency in allergy soon finds himself busy with this type of condition alone. The next logical development is the allergy specialist who treats only allergic diseases. There are vast numbers of asthma, hay fever, and urticaria victims; and the exclusive allergist accomplishes untold good in relieving these suffering hosts.

But we should bear in mind that allergy is but a branch of medicine and that it must not always be considered as a disease apart from other medical maladies.

Balyeat[1] has indicated that the sufferer from allergy is likely to be otherwise in exceptionally good health and has suggested that in some way the allergic state may offer a measure of protection against some of the other diseases. The element of heredity appears to be linked up in this situation. Nevertheless, while the proportions may be smaller, my experienc has been that the allergic patient may be the victim of almost any of the other diseases which fall within the domain of internal medicine. I have not yet had an opportunity to make a statistical survey of my allergic series with this point in mind except with regard to blood pressure findings.

Hypotension is found in allergic patients often enough to have some diagnostic significance. Five per cent of my total series of office cases have hypotension. Twenty per cent of my allergic patients show low blood pres-

*Read before the Association for the Study of Allergy, Portland, Ore., July 9, 1929.

sure readings. But a larger proportion of my allergic patients have normal blood pressures and some even suffer from hypertension.

While startling therapeutic results are often obtained in the allergic diseases by specific allergic treatment or avoidance alone, there is a definite proportion of patients who do not respond to these methods. The writer[2] and Peshkin[3] have called especial attention to these groups and emphasized that other pathologic factors, nonallergic, may influence the allergic reaction and its response to treatment. In connection with this I proposed a term, "allergic balance" or "allergic equilibrium" to designate the state of an allergic individual who although in contact with an allergen to which he is sensitive, remains symptom free. It has been shown that this allergic balance may be overthrown, with the precipitation of an allergic attack, either by the addition of more extensive allergic contacts with one or several allergens, or by the activity of some additional nonallergic factor.

The concept which I wish to bring out in this contribution differs distinctly from the foregoing in that the disease from which the patient is suffering is primarily a nonallergic organ or systemic disease. An allergic reaction which would otherwise be insignificant with this patient or might not even give rise to symptoms, will increase the disability from the primary nonallergic disease. The first case to be cited will be open to argument in that there may be a question as to which is the dominant disease manifestation, the allergic or the nonallergic; but the others appear to be clear-cut examples of the effect of allergic reaction on the course of nonallergic disease.

CASE 1.—Mrs. W. P., aged twenty-six years, complained of eczema of the hands. She was tested out with the various food and epidermal proteins and gave borderline reactions only to the proteins of sweet potato, squash, onion, cottonseed, wheat, and milk. After nearly three months of protein elimination reinforced by nonspecific methods, such as peptone injections, hypertonic saline intravenously and acinotherapy, there was no definite improvement, and a basal metabolism determination was made. This test was done not because the patient was presumably an allergic who had not responded to other methods but because she looked like a hypothyroid case. Cerebration was a little slow, the hair was rather coarse, and the skin was definitely thick and pasty, giving a low-grade myxedematous appearance to the face.

The metabolic rate was found to be minus nine, around the lower limits of normal. The patient was placed on increasing doses of thyroid extract, running up to four grains daily; and her metabolic rate increased on successive readings to minus seven, plus five, and plus sixteen. Within ten days after the institution of thyroid therapy the eczema commenced to improve and in the third week it had cleared up entirely.

Through this time she had remained on her protein avoidances. After the hands had been free from eczema for over two weeks, she started using a prepared lard in cooking, one which contained cottonseed oil. The following day her eczema had returned. She then changed to pure hog lard and the hands promptly cleared up. She has had one recurrence since that time, following the use of a different soap which may have been made from cottonseed oil.

Here is a case of hypothyroidism in which allergic eczema followed contact with specific allergenic proteins. Dermatitis occurs not infrequently in hypothyroidism, and this raises the question whether the dermatitis is an integral part of the hypothyroidism or whether it may at times be due to a concomitant allergy. The man who is treating dermatitis in a myxedema case, with thyroid extract, must bear in mind that allergy may be a factor.

CASE 2.—Miss P. M. History of scarlet fever in childhood. No allergic history. At the age of twenty-three this patient's blood pressure was found to be elevated. The course of her illness followed in general that of other similar cases. For a few years she had a fluctuating hypertension. By the age of thirty-one it had become quite fixed around 170 and gradually increased to around 190 to 200. Occasionally it was higher. There was only moderate arteriosclerosis but considerable cardiac hypertrophy. By the time she was thirty-four she suffered her first acute cardiac upset with circulatory collapse, extrasystoles, and pulsus alternans. After a period of rest she was again back on her feet, but within thirteen months she began to have attacks of nocturnal dyspnea.

These appeared to be primarily cardiac. She would awaken suddenly with gasping and belching. There was no premonitory cough, no typical asthmatic wheezing, and there was not the typical asthmatic history of relief with the raising of increased amounts of mucus. The pulse was rapid but regular during the attacks. They passed off fairly rapidly, after the belching of large quantities of gas.

Although the case appeared to be a clear-cut example of true cardiac asthma, sensitization tests were performed and the patient was found to be clearly sensitive to feathers. She was also found sensitive to the proteins of egg, corn, mackerel, lobster, and mustard. She substituted silk floss pillows for feather pillows and remained free from these attacks of apparently cardiac asthma for nearly four months.

At the end of this time cardiac failure commenced. The systolic blood pressure fell from its level around 200, down to 138. The gas attacks at night recurred and were accompanied by cardiac pain and palpitation. They came with increasing frequency so that five months later she was practically confined to her bed with congestive heart failure and attacks of acute pulmonary edema. She died in her thirty-seventh year.

The attacks of acute pulmonary edema were altogether typical. They began with palpitation and shortness of breath, and sometimes precordial pain, which within a few minutes was followed by the raising of large amounts of frothy, bloody, serous fluid. This sputum was not at all that of an asthmatic. It is of especial interest that ephedrin always promptly relieved the attacks. Ephedrin had not, so far as I know, previously been tried out in acute pulmonary edema, but I have since used it in a case in which allergy did not appear to play a part, without benefit.

The generally accepted treatment of acute pulmonary edema due to cardiac failure consists of the injection of morphine and atropine. Adrenalin has been recommended, but some authorities are very positive in the statement that adrenalin makes the condition worse.

This patient obtained much better results from ephedrin than from morphine and atropine.

These observations strongly suggested that an acute allergic reaction at times brought on attacks of acute pulmonary edema associated with cardiac failure. One additional incident lends strong corroborative evidence. This patient had observed that in one movie theatre in her home town, a rather small, poorly ventilated one, during the last few months before she became bedridden, she would within fifteen or twenty minutes develop acute shortness of breath, dyspnea, and palpitation, and would soon be coughing up bloody froth. This was the only building in which the phenomenon occurred. Indeed with this one exception the attacks occurred practically always at night after retiring. I had the opportunity of observing her during one of these attacks which came on at the movie and which rapidly cleared up within half an hour after leaving the place and within fifteen minutes after the administration of fifty milligrams of ephedrine by mouth. That the phenomenon was not associated with eye strain or some other possible factor is indicated by the fact that she could go into other cinemas without trouble. She was not sensitive to orris root.

CASE 3.—B. S., a girl seven years old, with moderately advanced pulmonary tuberculosis was under treatment at Blue Ridge Sanatorium, Charlottesville, Va. She was sensitive to timothy, orchard grass, and ragweed. She was admitted during the winter and manifested rapid improvement in her tuberculous infection. Serial x-rays showed corresponding evidence

of improvement. With the onset of the pollen season her asthma reappeared, accompanied for the first time in several months by fever. The next roentgenogram showed a definite reactivation, with increase in the distribution of the tuberculous process. Following subsidence of her asthma the temperature again returned to normal.

DISCUSSION

These 3 cases scarcely require prolonged discussion. Their significance appears to be self-evident. Certainly in the second and third, acute allergic reactions exerted a detrimental effect on the course of the chronic, nonallergic diseases. It seems reasonable to presume that other nonallergic diseases than those mentioned might also be so influenced.

Many observers have suggested that allergy plays a part in migraine. In 1927, I presented[4] the evidence from my clinical experience which seemed to demonstrate conclusively the presence of an allergic factor in migraine. There are some who maintain that biliary tract pathology or duodenitis or colonic disease or some obscure central nervous system defect, as the case may be, is the primary cause for migraine. Certain it is that in migraine there is often close association with biliary tract disease and probably also with duodenitis. Now it really makes no great difference which is the primary underlying pathology, allergy or the abdominal condition. Apparently one may influence the other.

In 1922, I described my first case of mucous colitis[5] in which there appeared to be an allergic factor. In 1927, Hollander[6] described 5 cases and, in 1928, I added 6 more to my own series.[7] In this communication I brought forth evidence that allergy is at least one factor in the causation of mucous colitis. I emphasized that certainly, at least in the well-developed cases, it is not the only factor, since such conditions as constipation, infection, food outrage, needless surgery, irritating enemas and the like serve to exaggerate the local pathology.

I have presented elsewhere what I consider excellent evidence that some cases of mucous colitis as well as migraine are primarily allergic. But for the present discussion it makes little difference whether they are basically allergic diseases, or fundamentally nonallergic. In either case allergy often colors the picture and not infrequently exaggerates the symptoms. But in the case of chronic myocarditis and chronic pulmonary tuberculosis, the allergic reaction appears to be extrinsic to the basic pathology. Sensitization to the protein of the tubercle bacillus need not enter into the present discussion.

In conclusion, I would say that this paper is presented, first as a plea to the man who is limiting himself strictly to allergy, calling his attention to the possibility of allergic factors in other maladies; and second, as a plea to the general physician and other specialists, reminding them that inasmuch as 10 per cent of all people appear to be allergic in one way or another, allergy is a condition which sometimes must be brought into consideration in the treatment of apparently nonallergic diseases.

Allergy is not strictly speaking a specialty. Like tuberculosis, cardiology, and gastroenterology, it is but a subdivision of internal medicine, and for most useful accomplishment must not be too widely separated from the more general subject.

Balyeat[8] has placed before us the need for more thorough undergraduate instruction in clinical allergy in our medical schools. The cases here reported illustrate how an internist might serve his patients better by virtue of a more thorough understanding of allergy and its synergistic potentialities.

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THE RÔLE OF BACILLUS WELCHII IN PERNICIOUS ANEMIA*

By W. Leonard Forsyth, M.B., D.P.H. (Lond.), and Y. B. Abdoosh, M.B., Cairo, Egypt

FOR many years the intestinal canal has been searched for the cause of pernicious or idiopathic anemia. Various organisms have been considered and rejected. Most important of these are the B. coli and streptococcus groups and more recently anaerobes, particularly Bacillus welchii. A possible etiologic relationship has been inferred from the greatly increased numbers of these organisms in the intestinal tract, and their appearance at higher levels in the upper small intestine, which is relatively free from bacteria in the healthy individual.

In the *Journal of Pathology and Bacteriology*, July, 1928, Davidson shows that very high counts of B. welchii are obtained in this disease. He employs a fecal suspension of definite opacity in decimal dilutions ranging from 1 in 10 to 1 in 100 million, distributed in Tubes No. 1 to No. 9. These tubes are plated to a ferrous sulphate-sodium sulphite glucose agar medium for the growth of anaerobic bacilli in the vegetative stage. Anaerobes reduce the sulphite to sulphide, producing black colonies. We have examined a large number of these colonies and confirm Davidson's finding, that a large proportion of black colonies (over 90 per cent) contain B. welchii. He finds that Tube 3 (dil. 1 in 100) represents the upper limit from which B. welchii is isolable in healthy persons, while in 33 of 41 patients suffering from pernicious anemia, B. welchii was isolated from Tube 9.

His technic has been closely followed for the sake of comparison in a survey of the feces of healthy Egyptians. Over one hundred prisoners in a local jail, in good health, on a homogeneous diet, largely vegetable, and having had no antecedent bowel complaint were the subject of examination. The B. welchii content only was considered.

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We have found that 21 per cent of these show B. welchii in very high dilutions, i.e., between Tubes 3 and 9, and most of them in and nearer 9 than 3. In other words they are in such quantity as to occupy a pathologic zone in terms of Davidson's standard.

A point of interest in this connection is that pernicious anemia is a very uncommon disease in Egypt compared with its incidence in England.

Recently one of us (W. L. F.) has investigated the stools of healthy Egyptians in relation to their content of B. tetani and found these exceedingly rich in their yield of this organism.

We would conclude that the stools of healthy Egyptians have an anaerobic standard different from that reported for others by Davidson.

STUDIES IN THE PHARMACOLOGY OF LOCAL ANESTHETICS*

III. Comparison of Gamma-(2-methyl piperidino)-propyl Benzoate Hydrochloride with Cocaine and Procaine on Experimental Animals

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INTRODUCTION

PRELIMINARY pharmacologic data on certain of McElvain's[1] local anesthetics have been reported by Jones[1, 2] and Rose,[3, 4, 5, 6, 7] and published by McElvain and his associates. Further physiologic investigations show several of these compounds to be outstanding when toxicity, duration of anesthesia, lack of irritating properties, and potentiation by adrenalin are considered. They are soluble in water, their solutions stable, and they are readily sterilizable by heat. After it had been ascertained that they possessed the properties of a good local anesthetic,[8] they were submitted to Dr. Wm. R. Meeker of Mobile, Alabama, for comparative evaluation. Dr. Meeker conducted the tests upon himself, and in a personal communication stated that gamma-(2-methyl piperidino)-propyl benzoate hydrochloride (hereafter designated as No. 33) was the best of those studied by him.

Further and more detailed study of this one compound was required. Some of the results of that work are presented here. To obtain an idea of the value of No. 33 in comparison with substances with which the clinicians have had wide experience, a number of pharmacologic tests have been made, using cocaine and procaine parallel with the new local anesthetic. The data included are results obtained from this work.

METHODS OF EVALUATION

Duration of anesthesia and toxicity are the chief factors influencing the value of any local anesthetic substance. Toxicity data involve only animal

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experimentation whereas duration has been determined by the use of both animal and human subjects. To prevent confusion, only the work done on animals will be included in this article. Clinical data and the accumulated experiments on human subjects will appear later.

1. *Duration of Anesthesia.*—The duration of anesthesia on mucous membranes was determined by the method described by Schmitz and Loevenhart.[9] An aqueous solution of the drug to be tested was instilled into the pouched lower lid of a rabbit's eye and the cornea bathed for one minute. After the remaining solution was drained off, stimulation was given the cornea by application of a dull-pointed glass rod. No effort was made to distinguish between partial or deep anesthesia, duration being judged as the time intervening between the loss of the wink reflex and its return.

Intracutaneous infiltration duration was determined by the method described by Rose.[10] Injections of given doses of solutions of equal percentage were made on the closely clipped backs of guinea pigs. The wheals thus formed were stimulated by means of an induced electric current. Any movement of the animal due to the stimulation was considered the end-point of duration.

2. *Toxicity.*—Intraperitoneal toxicity values on rats were the result of injections made halfway between the sternum and the symphysis pubis in the midline. This test was done in an attempt to obtain some idea of the toxicity of these compounds when used in the urinary tract, since the vascularity of the peritoneum and its contents most nearly approaches that of the urethra and bladder. A direct determination by injection into the urinary tract of experimental animals was not practical because it could not be done without operative procedure. This would have involved the use of general anesthesia, a factor which influences the toxicity of local anesthetics greatly.

Intravenous toxicity experiments were done on rats and rabbits. For the rats, the intravenous injection method of Roth[11] was used. Injections were made at the rate of fifteen to eighteen seconds for every one-tenth of a cubic centimeter of solution,[12] in order that the speed of the injection might not be a factor in increasing the toxic effect of the local anesthetics. The same point is of extreme importance in every case of intravenous injection and was observed very carefully in the work with rabbits. The lateral marginal vein of the ear was the portal of entry in the case of this animal.

Subcutaneous injections for toxicity determinations on mice were done after the method of Schmitz and Loevenhart.[13] The injections were made in the abdominal region as near the lower margin as possible and in volume doses never exceeding one-half of a cubic centimeter. The same general method was followed in the case of white rats.

DATA

Table I shows the duration of anesthesia upon the rabbit's cornea when the local anesthetic is in concentrations of 1 and 2 per cent in aqueous solutions, and the duration of anesthesia intracutaneously in the guinea pig, in doses of one-tenth of one cubic centimeter of a 1 per cent solution.

For the rabbit's cornea anesthesia, values given here for cocaine correspond well (within experimental limits) to those already recorded. The literature gives for 1 per cent solutions: eighteen minutes,[18] eighteen and twenty minutes,[16] and for 2 per cent solutions: twenty-seven minutes,[16] twenty-eight minutes,[13] twenty-nine minutes,[3, 24] thirty minutes,[18] and thirty-two minutes.[19]

TABLE I*

DURATION OF ANESTHESIA (EXPRESSED IN MINUTES)

ANESTHETIC	INTRACUTANEOUS INFILTRATION GUINEA PIGS	RABBIT'S CORNEA	
	1%	1%	2%
Cocaine	31	24	35
Procaine	24	no action	no action
No. 33	44	22	34

*The figures for this table are based on the results obtained from not less than 20 animals in each case. For cocaine, 1 per cent solution on rabbit's cornea, the number of animals used was 120. 2 per cent solution, 24. Six of the latter group gave the duration value of sixty minutes, reported in the second paper of this series. (J. LAB. & CLIN. MED. 15: 239. 1929.)

Cocaine anesthesia by subcutaneous injection on a dog's back is reported by Pittenger,[17] as being eleven times more effective than procaine. Meeker,[19] using the same method and doses of 0.25 c.c. of 1 per cent solutions, found the duration of cocaine anesthesia to be thirty minutes, and procaine, eighteen minutes. In the concentrations used, and when in contact with the rabbit's cornea for one minute, procaine produces little or no anesthesia.

The data found in Table II are toxicity test results expressed in terms of the "median lethal dose." Considerable confusion seems to exist as to the meaning of "minimal lethal dose" so long employed as an expression of toxicity. It is considered by some authors as the amount of a drug just sufficient to kill an animal occasionally, that which kills 50 per cent, or that which is just large enough to kill all the animals on a given dose.[20] The term "median lethal dose," which means the dose that will kill 50 per cent of a *large** group of animals, has been decided upon as giving the fairest measurement of toxicity values. This term together with its abbreviation L. D. 50 (lethal dose, 50 per cent) was suggested by Trevan.[20]

TABLE II

TOXICITY DATA IN TERMS OF L. D. 50

METHOD OF INJECTION	ANIMAL	COCAINE	PROCAINE	NO. 33
1. Intravenous	Rats	17.5	53	20
2. Intravenous	Rabbits	17.0	57	28
†3. Subcutaneous	Mice	150.0	800	800
†4. Subcutaneous	Rats	250.0	2100	1300
5. Intraperitoneal	Rats	70.0	300	120

†The subcutaneous injections in the case of mice and rats were repeated with adrenalin in dilution of 1-25000 [14], added to the anesthetic solutions. The L. D. 50 for cocaine, procaine, and No. 33 with adrenalin, on mice was 150, 800, and 900 milligrams per kilogram, respectively. On rats the results were (in the same order) 110, 2600, and 1500 milligrams per kilogram.

The values given above for cocaine and procaine agree within the limits of experimental error, with those reported in the literature to date. Cocaine

*Not less than 10 animals in each group.

toxicity per kilogram of body weight by intravenous injection on white rats is reported as 12.5 mg.[21, 22] and 17.5 mg.[3]; and on rabbits, 7.5 mg.[23] injected fairly rapidly.

Toxicities for cocaine by subcutaneous injections on white mice are reported as 150,[13, 24] 200-350[23] and 250 mg. per kilogram[3]; and on rats, 200-300[23] and 250 mg. per kilogram.[13]

For procaine, the intravenous injection results recorded on rats are 50,[3] and 45 to 55 mg. per kilogram;[21, 22, 23] and on rabbits, 40 to 70[23] and 55 to 65 mg. per kilogram.[15]

Toxicities, by subcutaneous injection of procaine on mice, are stated by other workers to be 900[24, 13] and 1,000 mg. per kilogram;[3] and on rats, 1600 to 2000[23] and 1600 mg. per kilogram.[13]

THERAPEUTIC INDICES

Therapeutic indices were calculated according to the method and formula of Schmitz and Loevenhart,[9] as follows: The numerical duration value of a given local anesthetic multiplied by the numerical toxicity value of the same substance is divided by a number derived in the same way from the standard. Cocaine taken as the standard has the value of unity. The other substances which are to be compared may be referred to in terms of cocaine.

Letting "R" represent the anesthetic which is to be compared with the standard "S," the formula reads as follows:

$$\frac{\text{R duration of anesthesia by R toxicity}}{\text{S duration of anesthesia by S toxicity}} = \text{Therapeutic Index}$$

All data in Table III were derived from Tables I and II.

TABLE III

THERAPEUTIC INDICES

ANESTHETIC	METHOD	PER CENT SOLUTION	NUMBERS CORRESPOND TO TESTS IN TABLE II					
			1	2	3	4	5	AVERAGE
Cocaine	Rabbit's cornea	1	1.00	1.00	1.00	1.00	1.00	1.00
No. 33	Rabbit's cornea	1	1.05	1.51	4.90	4.77	1.57	2.76
Cocaine	Rabbit's cornea	2	1.00	1.00	1.00	1.00	1.00	1.00
No. 33	Rabbit's cornea	2	0.75	1.60	5.18	5.05	1.66	2.85
Cocaine	Guinea pig	1	1.00	1.00	1.00	1.00	1.00	1.00
No. 33	Guinea pig	1	1.62	2.34	7.56	7.38	2.44	4.27
Procaine	Guinea pig	1	2.52	2.60	4.13	6.51	3.22	3.80

SUMMARY*

By Table I it is shown that No. 33 is equally as active as cocaine in 1 and 2 per cent solutions on the rabbit cornea, and twice as active as procaine and one and one-half times as active as cocaine by the guinea pig infiltration method.

Table II shows that No. 33 is less toxic than cocaine in every case using five different methods of administration. It is more toxic than procaine by all methods. Subcutaneously on rats it shows a wide margin of safety.

The therapeutic indices derived from the data in Tables I and II and shown in Table III give the advantage to No. 33 over cocaine in 1 and 2 per

cent solutions on the rabbit's cornea in nine cases out of ten considered. By the guinea pig method, No. 33 has the advantage over procaine in two of five cases. It is to be noted however that the average of the five shows a result in favor of No. 33. The numerical values of No. 33 in this group of indices are higher than those of cocaine in every instance.

CONCLUSIONS

1. No. 33, gamma-(2-methyl piperidino)-propyl benzoate hydrochloride, has been compared pharmacologically with cocaine and procaine.

2. No. 33 is much less toxic than cocaine when given by subcutaneous injection.

3. While being more toxic than procaine, No. 33 still has a great margin of safety when administered subcutaneously.

4. No. 33 is active by topical application to mucous membranes as well as by infiltration.

5. No. 33 compares favorably with the recognized local anesthetics, cocaine and procaine.

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LABORATORY METHODS

THE TECHNIC OF DETERMINATION OF THE RELATIVE MASS, THE INDIVIDUAL CELL VOLUME, AND THE VOLUME INDEX OF THE ERYTHROCYTES OF MAN*

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THE determination of the size of the erythrocyte is of increasing importance in clinical medicine. Many examples of the value of such data might be given. To mention only one, in pernicious anemia, an accurate determination of the size of the red cells is necessary for diagnosis and the one most valuable indicator of the course of the disease. The widespread interest in the subject of red cell size is best shown by the increasing amount of research concerning it.

One measure of the size of the erythrocyte is its relative diameter. The pioneer work of Price-Jones[1] on the red cell diameter in health and disease has stimulated much research from this angle. The measurement of diameter only does not take into account the thickness of the cell, and relatively few cells are measured in practical clinical work. It seems far preferable to determine, as a measure of size, the total cell volume since this includes all dimensions.

The determination of volume involves first of all an accurate estimation of the relative mass of corpuscles in a given volume of blood, or of the volumetric relationship between corpuscles and plasma. The relative mass is the number of c.c. of corpuscles per 100 c.c. of blood when the corpuscles without alteration in volume are packed closely so that no plasma remains in the interstices of the cells.

The red corpuscles are composed of a semisolid hemoglobin-containing material which at the surface is dense and membrane-like. They are elastic and flexible, changing form and volume easily under different conditions. Variations in the electrolyte content of the surrounding medium quickly cause changes in the cell volume. Proper packing to eliminate fluid between the cells requires sufficient time and power of centrifugation.

The estimation of the average individual cell volume necessitates also an accurate count of the red cells of the blood for which the relative cell mass is being determined. The volume index is only a comparison of the individual cell volume of the unknown blood with the known normal individual cell volume. The two procedures involved are the estimation of the relative mass per 100 c.c. or the volume percentage of red cells and the estimation of the number of red cells per cm.

The relative cell mass is determined by the rapid centrifugation of unaltered blood or by centrifugation for a longer period of time of blood to which an anticoagulant has been added. The true volume is obtained only when the individual cell volume is unaltered and packing is complete. Numerous methods

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have been employed in determining the cell volume but few meet these requirements.

I have summarized in Table I all the more important investigations on the determination of the red cell volume of man. Many other studies have been made but are not sufficiently complete to justify inclusion. The volume percentage of cells is much the same everywhere when determined by an accurate method. The number of red cells vary quite widely so the average individual cell volume shows quite marked variations. The summary in Table I shows the figures for men for different workers when an isotonic coagulant is used or when the necessary corrections are made for shrinkage due to use of a hypertonic anticoagulant.

Van Allen[15] in describing a new hematocrit tube reviewed the important factors in the accurate determination of the red corpuscle volume. He found 1.3 per cent sodium oxalate isotonic with the blood of the rabbit but reported no studies with human blood. He used a capillary hematocrit in a centrifuge of 9 cm. radius and centrifuged for fifteen minutes at 2700 r.p.m.

The different factors to be considered in the determination are: (1) type of hematocrit; (2) the anticoagulant; (3) the time and power of centrifugation.

TYPE OF HEMATOCRIT

The earlier investigators employed the hematocrit invented by Blix and first described by Hedin.[16] This is a straight capillary tube about 10 cm. in length which is filled with unaltered blood and centrifuged at high speed for a few minutes. Only a small amount of blood is required and no anticoagulant is necessary. This is neither a satisfactory nor an accurate method and is now seldom used. The only extensive research work done with this instrument was that of Capps in 1905.

The Bönninger tube is a modification of the hematocrit of Hedin. It is a graduated capillary U-tube which is filled with blood to which an anticoagulant has been added. This was first used by Bönninger,[4] and later by Csaki,[5] and by Froelich.[7] I have had no experience with this tube but see little to recommend it since a larger tube is far preferable.

Several other sizes of straight capillary tubes have been employed. These have usually been made and graduated in the laboratory in which they have been used. In addition to not being standard instruments these have all the disadvantages of the Hedin tube.

The best of the capillary tubes is that devised by Van Allen.[15] This enables the use of a standard centrifuge and fittings with an isotonic anticoagulant solution. It has the disadvantages of other micro methods. Results with this tube are not included in Table I since no data have been reported for human blood.

In my original work on volume index[10] I employed a graduated 15 c.c. centrifuge tube using 10 c.c. of blood and 2 c.c. of the anticoagulant solution. This is a standard tube, can be easily accurately calibrated and used with the usual centrifuge fittings. It has the disadvantage of requiring 10 c.c. of blood and the results cannot be read quite so sharply as with a tube of smaller caliber. Kuhnel,[11] Osgood,[12] and Wintrobe and Miller[14] used graduated tubes of 4 or 5

TABLE I

SUMMARY OF RESULTS OF DIFFERENT WORKERS ON DETERMINATION OF VOLUME OF THE RED BLOOD CELL

NAME OF OBSERVER	DATE	COUNTRY	TYPE OF HEMATOCRIT	TIME OF CENTRIFUGATION	ANTICOAGULANT	AVERAGE RED BLOOD CELL VOLUME PER 100 C.C. (= VOLUME PERCENTAGE OF CELLS)	AVERAGE RED BLOOD CELL COUNT	AVERAGE RED BLOOD CELL VOLUME FOR 5 MILLION CELLS	AVERAGE VOLUME OF INDIVIDUAL RED BLOOD CELL
						c.c.	millions	c.c.	cubic microns
Capps[2]	1903	United States (Boston)	capillary tube (Hedin)	3 minutes at 10,000 r.p.m.	none	men (4) and women (6) 48.0	4.83	50.00	100.00
Larrabee[3]	1911	United States (Boston)	capillary tube (2 mm. x 10 cm.)	gravity sedimentation for 1-3 days	100 mg. sodium oxalate to 10 c.c. blood	men (12) 53.0 women (9) 49.7	5.27 4.97	50.00	100.00
Bönninger[4]	1919	Germany	capillary tube (Bönninger)	to constant volume (40 minutes) at 4000 r.p.m.	hirudin	men (10) 46.4 women (10) 39.4	4.87 4.30	46.8	93.6
Csaki[5]	1922	Hungary	capillary tube (Hedin)	7 minutes	none	men (11) 45.2 women (12) 44.0	4.94 4.81	45.8	91.6
Campbell[6]	1922	England	capillary tube (size ?)	15 minutes	50 mg. potassium oxalate to 10 c.c blood	men and women (16) 40.00	5.15	38.8	77.6
Froelich[7]	1922	Germany	capillary tube (Bönninger)	30 minutes at 4000 r.p.m.	hirudin	men (7) 45.0 women (13) 41.4	4.96 4.48	45.8	91.6
Bie and Möller[8]	1922	Norway	capillary tube (1 mm. x 5 cm.)	½-1 hour	defibrinated	men (10) 46.4 women (10) 38.7	5.53 4.74	41.2	82.6
Gram and Norgaard[9]	1923	Norway	capillary tube (10 cm.)	until transparent (1-1½ hours) at not less than 3000 r.p.m.	hirudin	men (10) 46.3 women (10) 40.5	5.45 4.65	43.4	86.8

*When corrected for shrinkage due to oxalate (6.7%) this figure becomes 46.5 c.c.
†When corrected for shrinkage due to oxalate (3.5%) this figure becomes 46.6 c.c.

TABLE I—CONT'D

NAME OF OBSERVER	DATE	COUNTRY	TYPE OF HEMATOCRIT	TYPE OF CENTRIFUGATION	ANTICOAGULANT	AVERAGE RED BLOOD CELL VOLUME PER 100 C.C. (= VOLUME PERCENTAGE OF CELLS)	AVERAGE RED BLOOD CELL COUNT	AVERAGE RED BLOOD CELL VOLUME FOR 5 MILLION CELLS	AVERAGE VOLUME OF INDIVIDUAL RED BLOOD CELL
						c.c.	millions	c.c.	cubic microns
Haden[10]	1923	United States (Kansas City)	15 c.c. graduated centrifuge tube	30 minutes at 2500 r.p.m.	2 c.c. 1.6% sodium oxalate to 10 c.c. blood	men (40) 47.7 women (12) 41.0	4.98 4.26	48.0	96.0
Kühnel[11]	1925	Denmark	5 c.c. graduated tube	to constant volume (30 minutes)	1 c.c. 3% sodium citrate to 9 c.c. blood	women (10) only 41.5	4.74	43.7	87.4
Osgood[12]	1926	United States (Portland, Oregon)	4 c.c. graduated tube	to constant volume (½-1 hour)	20 mg. potassium oxalate to 10 c.c. blood	men (94) 45.0† women (100) 41.0	5.40 4.80	43.0	86.0
Jorgensen and Warburg[13]	1927	Denmark	thin tubes (size ?)	until transparent at 4000 r.p.m.	defibrinated	men (3) and women (4) 45.5	5.30	43.0	86.0
Wintrobe and Miller[14]	1929	United States (New Orleans)	4 c.c. graduated tube	to constant volume (½-1 hour) at 3500 r.p.m.	40 mg. potassium oxalate to 10 c.c. blood	men (100) only 43.4*	5.85	37.1	74.2
Haden	1929	United States (Kansas City)	12 c.c. graduated centrifuge tube	1 hour at 2500 r.p.m.	2 c.c. 1.4% sodium oxalate to 10 c.c. blood	men (7) and women (3) 44.8	4.86	46.1	92.2

TABLE II

	NO. CASES	LOCATION	RED BLOOD CELLS PER 100 C.C. (= VOLUME PERCENTAGE OF CELLS)
Bönninger	10	Germany	46.4 c.c.
Csaki	11	Hungary	45.2
Froelich	7	Germany	45.0
Bie and Möller	10	Norway	46.4
Gram and Norgaard	10	Norway	46.3
Haden	20	United States	46.5
Osgood	94	United States	46.6
Jorgensen and Warburg	3	Denmark	45.5
Wintrobe and Miller	100	United States	46.5

c.c. capacity made from Mohr pipettes. These require smaller amounts of blood, but are not standard and require special calibration.

There is never any objection, even in the most anemic patient, to taking sufficient blood for a careful study. A macro method is necessary for accuracy. Specially made tubes of 4 or 5 c.c. capacity when accurately calibrated are perfectly satisfactory. I still prefer to use a standard 15 c.c. centrifuge tube with 10 c.c. of blood and 2 c.c. of isotonic anticoagulant. Here one is using sufficient blood for accurate reading in a standard piece of apparatus. 12 c.c. centrifuge tubes of pyrex glass are now available also and are somewhat more satisfactory than those of 15 c.c. capacity. I have used these 12 c.c. tubes exclusively in the experiments reported herewith. Each tube was calibrated by weighing with distilled water.

THE ANTICOAGULANT

The original Blix hematocrit was designed for use without an anticoagulant. No anticoagulant was used by Capps. There are only two anticoagulants which do not change the volume of the red cell and yet add no volume to the blood. These are heparin and hirudin. Hirudin was used by Bönninger, by Froelich, and by Gram and Norgaard.[9] The other anticoagulants used are citrate and oxalate. Three per cent sodium citrate was used by Kühnel and by de Jong.[17] The oxalates have been used in a number of ways. A method in common use is the addition of one drop of a saturated solution of potassium oxalate to each 5 c.c. of blood. Twenty mg. of solid potassium oxalate per 10 c.c. of blood is often recommended. Campbell[6] used 50 mg. of solid potassium oxalate to 10 c.c. and Wintrobe and Miller 40 mg. to 10 c.c. Larrabee[3] used 100 mg. of solid sodium oxalate to 10 c.c. of blood.

Hooper, Smith, Belt, and Whipple[18] pointed out that solid oxalate causes a marked shrinking of red cells. They suggested the use of 2 c.c. of a 1.6 per cent solution of sodium oxalate to 10 c.c. of blood for determining the volume percentage of red cells in the dog. This method was used in my original work in determining the volume index of the red cells of man. Later observers who have used solid potassium oxalate have recognized the fact that the cell volume is decreased with this procedure. Osgood states that the use of 20 mg. to 10 c.c. causes a 3.5 per cent shrinkage. Wintrobe and Miller allow 6.7 per cent for shrinking on adding 40 mg. of oxalate to 10 c.c.

It is apparent that it is most unsatisfactory to attempt accurate results unless the cell volume is unchanged. I have made a series of experiments with human blood to determine the variation in volume with different anticoagulants. There were 100 c.c. of blood withdrawn with a large syringe from each individual and just enough hirudin or heparin added to prevent coagulation. With a calibrated pipette, 10 c.c. of blood was run into each of a series of 12 c.c. centrifuge tubes. The pipette and tubes were accurately calibrated by weighing with water on a chemical balance. One tube was taken as a control. To the other tubes the different anticoagulants were added as indicated. All tubes from one individual were centrifuged simultaneously as indicated in the tables.

In the initial experiment (Table III) different dilutions of sodium oxalate and one drop of a saturated solution of potassium oxalate were used. These showed the marked shrinkage due to use of the saturated solution of potassium oxalate. Two specimens of defibrinated blood gave results slightly higher than with hirudin alone. It is apparent from this preliminary experiment that the correct amount of sodium oxalate to prevent coagulation without changing the cell volume lies between 1.4 per cent and 1.6 per cent.

TABLE III

VOLUME PERCENTAGE OF RED BLOOD CELLS WITH DIFFERENT ANTICOAGULANTS. (CENTRIFUGATION FOR ONE HOUR AT 2500 R.P.M.)

NO.	VOLUME PERCENTAGE OF RED BLOOD CELLS WITH							
	HIRUDIN ONLY	10 C.C. HIRUDINIZED BLOOD WITH 2 C.C. SODIUM OXALATE					10 C.C. WITH 1 DROP SATURATED SOLUTION POTASSIUM OXALATE	DEFIBRINATED ONLY
		1.2%	1.4%	1.6%	1.8%	2.0%		
1	44.5	46.0	45.0	44.0	43.0	43.0	41.0	47.0
2	44.0	45.0	44.0	43.0	43.0	42.5	38.0	45.0
3	49.0	50.0	50.0	48.5	47.5	46.5	44.0	—
Average	45.8	47.0	46.3	45.2	44.5	44.0	41.0	—

In the second set of experiments (Table IV) only three dilutions of sodium oxalate were used, namely 1.4, 1.5, and 1.6 per cent. The effect of adding solid sodium oxalate equivalent to that as contained in 2 c.c. of a 1.4 per cent solution was determined by adding 28 mg. to 10 c.c. of blood. Other tubes contained 1 c.c. of a 3 per cent solution of sodium citrate, 20 mg. of solid potassium oxalate, or 1 drop of a saturated solution of potassium oxalate. The cell volume with the solid sodium oxalate and potassium oxalate, and the saturated solution of potassium oxalate are much the same and are all much lower than with the hirudinized blood. The readings with the 1.4 per cent sodium oxalate solution (2 c.c. to 10 c.c. of blood) are almost exactly the same as with hirudinized blood.

These observations together with succeeding ones have convinced me that the 1.4 per cent sodium oxalate is isotonic with human blood since it gives the same results as heparin or hirudin. On the other hand the addition of 1 drop of a saturated solution of potassium oxalate (equivalent to 25 mg. of oxalate) to 10 c.c. of blood causes the volume of cells to shrink 8 per cent.

TABLE IV

VOLUME PERCENTAGE OF RED BLOOD CELLS WITH DIFFERENT ANTICOAGULANTS (2500 REVOLUTIONS PER MINUTE)

NO.	TIME OF CENTRIFUGATION HOURS	HIRUDIN ONLY	10 C.C. HIRUDINIZED BLOOD WITH 2 C.C. SODIUM OXALATE			10 C.C. HIRUDINIZED BLOOD WITH 28 MG. SOLID SODIUM OXALATE	10 C.C. HIRUDINIZED BLOOD WITH 1 C.C. 3 PER CENT SODIUM CITRATE	10 C.C. HIRUDINIZED BLOOD WITH 20 MG. SOLID POTASSIUM OXALATE	10 C.C. HIRUDINIZED BLOOD WITH 1 DROP SATURATED SOLUTION POTASSIUM OXALATE
			1.4%	1.5%	1.6%				
1	½	48.5	48.7	48.0	48.0	44.4	48.0	44.7	45.3
	1	47.5	48.2	47.0	47.0	44.4	47.0	43.7	44.3
	2	47.5	47.2	46.0	46.0	43.9	47.0	43.7	43.0
2	½	42.5	42.0	41.3	41.7	38.5	41.7	39.0	39.9
	1	42.0	41.0	40.3	40.7	37.0	40.7	38.0	38.4
	2	41.5	41.5	40.3	40.7	37.0	40.7	38.0	38.4
Average all readings		44.9	44.8	[illegible]	44.0	40.9	44.2	41.2	41.5

THE TIME AND FORCE OF CENTRIFUGATION

The time of centrifugation has varied widely with different workers. Unless an anticoabulant is employed, only a few minutes' packing is possible before coagulation takes place. It is necessary to pack the cells to constant volume. This usually requires about one hour. The centrifugal force of a centrifuge varies with the diameter and the speed. To compare different methods of centrifugation one must know both the speed and the length of the arm of the centrifuge. The centrifugal force of a centrifuge varies directly as the first power of the radius and as the second power of the speed. Thus the centrifugal force with the same r.p.m. with a radius of 20 cm. is twice that with a radius of 10 cm. If the radius is the same however, the centrifugal force is four times as great with a speed of 4000 r.p.m. as with a speed of 2000 r.p.m.

The striking thing shown in Table I however, is the relative constancy of the cell volume provided the proper anticoagulant is used even with varying methods of centrifugation. This suggests that complete packing does not necessitate great centrifugal force.

In determining the relative mass for calculating the volume index, it is only necessary to use the same procedure in each case. However, in calculating absolute numbers such as the individual cell volume it is necessary that no plasma remain in the interstices between the cells. This involves complete packing of the cells. This end-point is difficult to determine. The usual method has been to observe the cell volume of different intervals and continue the centrifugation until the volume is constant. Koeppe[19] thinks complete packing is best shown by translucency of the cell mass to transmitted light. This criterion has been utilized by some workers.[13]

I have made some experiments to determine the relation of the volume to the time and speed of centrifugation. The results are shown in Table V. All specimens were centrifuged and read at intervals of one-half, one, and two hours. The average of 12 determinations show the volume practically constant after an hour's centrifuging, so I have taken this time as the standard for comparison. The volume is only 2 per cent less with two hours' centrifugation than it is with centrifugation for one-half hour. The centrifuge used was an International No. 2 with a radius of 15 cm.

CALCULATION OF THE AVERAGE INDIVIDUAL CELL VOLUME

As early as 1867 Welcher[20] reported results on the size of the erythrocyte in terms of cubic microns. Numerous other workers have employed this most desirable unit of measurement. The only data needed for calculation are the relative cell mass and the red cell count. For instance from the data given in Table VI the average cell volume is calculated as follows: The average red blood cell count is 4.856 millions per c.mm. The volume percentage of cells with 1.4 per cent sodium oxalate solution is 44.82 c.c. equivalent to 44.82×10^{12} cubic microns. One hundred c.c. of blood with a red cell count of 4.856 million cells per c.mm. contains 4.856×10^{11} cells.

The average individual cell volume is

$$\frac{44.82 \times 10^{12}}{4.856 \times 10^{11}}$$ cubic microns or 92.1 cubic microns.

The individual cell volume in cubic microns is double the total cell volume per 100 c.c. of blood for 5 million cells per c.mm. For instance if the count is 4.0 million per c.mm. and 100 c.c. of blood contain 36 c.c. of cells the total cell volume for 5.0 million cells is 45, and the individual cell volume is 90.0 cubic microns.

TABLE V

RELATION OF TIME OF CENTRIFUGATION TO VOLUME PERCENTAGE OF RED BLOOD CELLS WITH DIFFERENT ANTICOAGULANTS

NO.	TIME OF CENTRIFUGATION AT 2500 R.P.M. (HOURS)	VOLUME PERCENTAGE OF CELLS WITH			AVERAGE
		HEPARIN ONLY	2 C.C. 1.4% SODIUM OXALATE TO 10 C.C. HEPARINIZED BLOOD	1 DROP SATURATED SOLUTION POTASSIUM OXALATE TO 10 C.C. HEPARINIZED BLOOD	
1	½	50.5	50.0	46.5	49.0
	1	50.5	50.0	48.5	48.7
	2	49.5	49.0	45.5	48.0
2	½	48.5	48.7	45.3	47.5
	1	47.5	48.2	44.3	46.7
	2	47.5	47.2	43.0	45.9
3	½	42.5	42.0	39.9	41.5
	1	42.0	41.0	38.4	40.5
	2	41.5	41.5	38.4	40.5
4	½	42.0	42.0	38.0	40.7
	1	42.0	41.0	38.0	40.3
	2	42.0	41.5	38.0	40.5
	½	Average of twelve determinations			44.7
	1	" " " "			44.1
	2	" " " "			43.7

TABLE VI

RELATION OF VOLUME PERCENTAGE OF RED BLOOD CELLS TO NUMBER OF CELLS (CENTRIFUGATION FOR ONE HOUR AT 2500 R.P.M.)

NO.	SEX	RED BLOOD CELL COUNT IN MILLIONS	VOLUME PERCENTAGE OF RED BLOOD CELLS WITH		
			HEPARIN ONLY	2 C.C. 1.4% SODIUM OXALATE TO 10 C.C. BLOOD	1 DROP SATURATED SOLUTION POTASSIUM OXALATE TO 10 C.C. BLOOD
1	M	4.80	44.0	44.0	40.5
2	M	4.83	44.5	45.5	41.5
3	M	4.93	47.0	47.5	42.0
4	F	4.30	40.5	40.0	38.5
5	F	4.38	42.0	41.0	38.0
6	M	5.45	50.5	50.0	45.5
7	M	5.60	47.5	48.2	44.3
8	F	4.64	42.0	41.0	38.4
9	M	4.85	47.5	47.0	42.5
10	M	4.78	44.0	44.0	42.0
Average		4.856	44.95	44.82	41.32

CALCULATION OF THE VOLUME INDEX

The volume index of Capps expresses the relationship between the number and size of the cells. It is calculated by dividing the volume percentage of cells in percentage of normal by the number of cells in per cent of normal. Example: The red cell count is 3,500,000 per c.mm. and 100 c.c. of blood contain 23.0

c.c. of packed cells. Normal blood with a count of 5 million contains 46.0 c.c. of cells per 100 c.c. The volume percentage of cells in the unknown blood is $\frac{23.0}{46.0} = 50$ (volume percentage of normal). The red cell count is 70 per cent of normal. The volume index is $\frac{50}{70} = 0.74$.

THE TECHNIC OF DETERMINATION OF THE RELATIVE CELL MASS

Run 2 c.c. of 1.4 per cent solution of sodium oxalate into a graduated 12 c.c. or 15 c.c. centrifuge tube with a syringe. Withdraw 20 c.c. of blood from an arm vein with a syringe. Transfer exactly 10 c.c. to the centrifuge tube containing the sodium oxalate solution and mix by inverting. Centrifuge for one hour at 2500 r.p.m. in a large centrifuge. Read the volume of cells. The blood of normal young men yields about 46 c.c. of cells and of young women about 41 c.c. To the other 10 c.c. of blood add one drop of a saturated solution of potassium oxalate and mix. This specimen is used for a red cell count and for hemoglobin determination.

ILLUSTRATIVE CALCULATIONS

On centrifuging 10 c.c. of blood mixed with 2 c.c. of 1.4 per cent sodium oxalate at 2500 r.p.m. for one hour, 4.5 c.c. of packed corpuscles are obtained. A normal blood with a count of 5 million yields 4.6 c.c. when similarly centrifuged. The volume percentage of normal of the packed cells of the unknown blood is 98.

The cell count of the unknown blood is 4,900,000.

The average individual cell volume is

$$\frac{45.0 \times 10^{12}}{4.90 \times 10^{11}} = 92.0 \text{ cubic microns}$$

The volume index is

$$\frac{\frac{4.5}{4.6}}{\frac{4.90}{5.00}} = 1.00$$

SUMMARY

Experiments are reported to determine the best method of estimating the volume relationship between the red corpuscles and plasma of men.

An accurate determination of this relationship necessitates the employment of an isotonic anticoagulant and sufficient power and time of centrifugation to completely separate the cells from the plasma.

A 1.4 per cent solution of sodium oxalate is isotonic with human blood.

Centrifugation for one hour at 2500 r.p.m. with radius of 10 cm. will completely separate erythrocytes from plasma.

The relative mass of the erythrocytes is best determined by centrifuging in such a way, 10 c.c. of blood to which 2 c.c. of 1.4 per cent sodium oxalate has been added. The average red cell content of the blood of young men is 46 c.c. per 100 c.c. of blood, and of young women 41 c.c.

Illustrative examples are given for the calculation of the volume index and the average individual cell volume.

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I. ON HEMOGLOBINOMETRY*

By Rawson J. Pickard, M.D., L. F. Pierce, Ph.D., and Robert L. Worthington, San Diego, Calif.

OF THE routine tests used in diagnosis, the estimation of hemoglobin, necessary in the diagnosis of some of the commonest diseases, is one of the most indefinite. The methods used have wide individual errors beside those inherent in technic, there are unnecessary errors in manufacture of the instruments, and there is a justifiable uncertainty as to the normal standard. The direct methods of hemoglobin determination are not suited to routine use. Of the two systems against which the clinical instruments should be standardized, the determination by the oxygen capacity method of van Slyke has many pitfalls, although it is shorter than the exact ferrometric methods.

The hemoglobinometers in general use in this country have been the Tallqvist, Sahli, and the Dare, and recently the Newcomer. The color scale of the Tallqvist on comparison with the Newcomer is more reliable than generally stated, provided the book is kept closed except at the moments of use. A hemoglobinometer resembling the Tallqvist sent out broadcast to physicians by a commercial house, advertising a remedy doubtless valuable in anemia is marked 10 per cent higher for each color than the Tallqvist. The Sahli is also a simple method and its readings appear somewhat more exact than the Tallqvist; its color solution also keeps well when kept in the dark, but there are wide errors in technic and in manufacture (irregular diameter of tubes).

There are several objections to the Dare hemoglobinometer. Newcomer[1]

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pointed out one of the most important—that there is no red glass whose light transmission curve at all corresponds to that of oxyhemoglobin, that to obtain an exact match a red light would have to be used, and that the sensitivity of the eye for red makes it impossible to distinguish any but gross differences in color. Any one familiar with the Dare has noted the differences in reading between different observers, due to their differences in color sensitivity, besides the difficulty of matching certain bloods, and the effects of fatigue for red. Another source of error with the Dare not previously recorded is the darkening of the blood by CO_2. While venous blood is rarely used with the Dare, capillary blood will invariably give higher readings than arterial, and especially is it higher in patients with poor aeration from any cause. After saturating blood with CO_2 we obtained readings with the Dare of 3.5 gm. Hb. instead of 2.7 gm. from the same specimen when oxygenated, with similar variations up to 15.15 gm. with CO_2 against 13.2 gm. oxygenated; 30 per cent to 15 per cent higher than arterial blood. In the Tallqvist the blood is immediately oxygenated, and this source of error does not exist. In addition, with the Dare there is the objectionable semisecret feature of the depth of the pipette, and the 15 per cent high reading for bloods over 65 per cent noted by Sanford, not present in all instruments, but seemingly common and due to insufficient grinding of the thicker half of the red glass prism. With these many sources of error it seems advisable to abandon the Dare hemoglobinometer.

The Newcomer glass disk has seemed the best hemoglobinometer for general adoption, being accurate, rapid, and not expensive, the B and L Newcomer costing little more than a Dare. By the purchase of a Newcomer disk alone at nominal cost, the Duboscq or other colorimeter in use in every laboratory can be used as a hemoglobinometer. Because of the close correspondence of its light transmission curve with that of acid hematin, the Newcomer disk is not only the best of clinical methods giving the same values for any eye but the only one giving readings approaching accuracy. It is not, however, an ideal method and has been modified several times. The original dilution of the blood was 1-250, the glass is now made to match against a 1-500 dilution (except the Klett instrument, 1-400). The original advice to take the average of ten readings because of the 5 to 10 per cent variation has unfortunately not recently been insisted upon. The difficulty of matching certain bloods because of color (icterus) or turbidity (fat, leucocytes) has been partly overcome by the addition by Bausch and Lomb of a dark blue glass filter, placed either in the lens system of the B. and L. hemoglobinometer, or laid on top of the eyepiece, when using a general laboratory colorimeter.

We had not questioned the general accuracy of the Newcomer disk until recently when we had occasion to examine a Klett colorimeter, with a dark glass disk, requiring, according to direction, a 1-400 blood dilution, with which, however, we got consistently higher readings. It is true, Klett says in the directions "it is probably well to recalibrate the glass plates," but we felt that this was a matter which the clinical laboratory had a right to leave to the manufacturer, and it is certainly not practical for the average physician who desires careful hemoglobin readings on his patients. We therefore made

readings of bloods, the hemoglobin of which we measured by the van Slyke oxygen capacity method, comparing the readings of the B and L Newcomer (two instruments), dilution 1-500, and Klett, dilution 1-400.

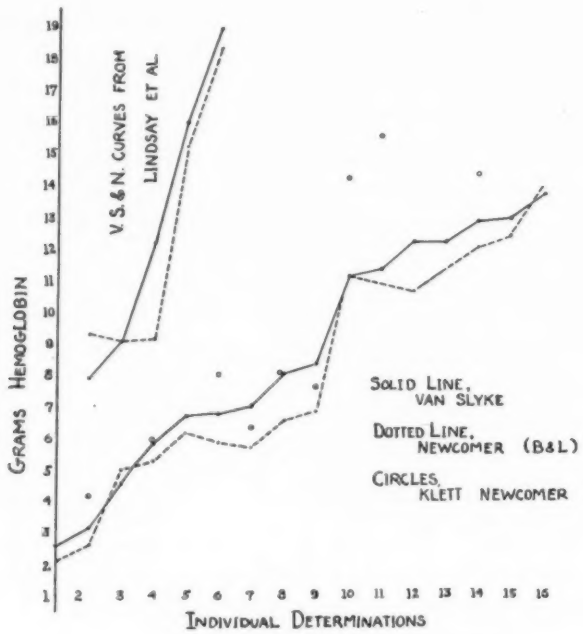

Chart 1.

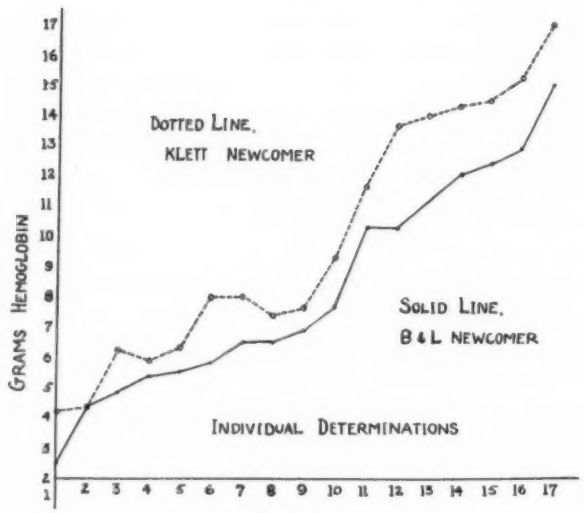

Chart 2.

Chart 1 shows our results. Chart 2 is a further comparison of the B. and L. Newcomer and the Klett. It is recommended by van Slyke[2] that his oxygen capacity method be repeated on a specimen until results are constant. This we did with only a few of the determinations reported in this study. A source of error is the settling of cells in the pipette, and this we found greater

in the pipettes with a long tip below the lower graduation, and in bloods diluted with saline instead of serum. On the whole the differences between the B. and L. Newcomer and the van Slyke in Chart 1 do not show any constant difference between the B and L Newcomer readings and the actual hemoglobin content, but rather the errors inevitable under ordinary working conditions, which are of wider range than we like to believe exist. Comparing our readings with those of Lindsay, Rice and Selinger[3] also given on Chart 1, we find their five readings total 63.8 for the van Slyke against 61 for the Newcomer; ours (sixteen) are 128.4 and 125.9 respectively, so close an agree-

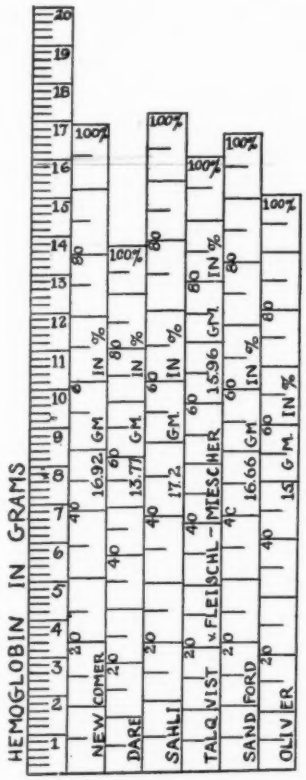

Chart 3.—Comparison of arbitrary standards of various hemoglobinometers, with accompanying hemoglobin scale for conversion of percentage values.

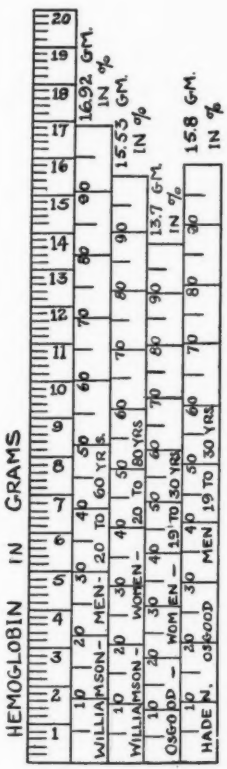
HEMOGLOBIN IN GRAMS
16.92 GM. IN %
15.53 GM. IN %
13.7 GM. IN %
15.8 GM. IN %
WILLIAMSON – MEN – 20 TO 60 YRS.
WILLIAMSON – WOMEN – 20 TO 60 YRS.
OSGOOD – WOMEN – 19 TO 30 YRS.
HADEN. OSGOOD MEN 19 TO 30 YRS.

Chart 4.—Normal adult hemoglobin standards with scale for conversion from grams to percentage or vice versa.

ment that we think with Senty[4] that the Newcomer (B. and L.) readings are so similar to the van Slyke that the Newcomer method is dependable, accurate, and "of great practical value in eliminating the preparation of standard solutions," provided the B. and L. disks are used, and provided it is recognized that there is a possible irregularity in individual determinations. The Klett disk was consistently too high in readings above about 9 gm. per cent, which would seem to indicate that the dark glass and 1-400 blood solution are wrong colorimetrically.

While the Newcomer hemoglobinometer is thus accurate for general clinical use, it must be remembered that it suffers from occasional erroneous

readings, a fault with many colorimetric methods, and is not dependable as a research method. Of these latter, all of which must first be standardized by the van Slyke or a ferrometric method, the Palmer method and that of Osgood and Haskins will doubtless give way to the photoelectric hemoglobinometer of Sanford and Sheard[5] which together with its accuracy has the great advantage of freedom from individual error. The Newcomer disk should be obtained from a firm guaranteeing its accuracy, unless standardization is to be undertaken in the laboratory. Readings with the Newcomer in cases in which the color index is important should be an average of ten readings from each of two blood dilutions. It is generally agreed that hemoglobin should be reported in grams per 100 c.c. Chart 3 is a convenient conversion table from the arbitrary standards of various instruments. It also gives the 16.66 gm. standard recommended by Sanford as convenient, in that 6 times the hemoglobin gives the percentage.

Williamson[6] using a spectroscopic method, measured the hemoglobin of 919 people, about 15 of each sex for each age period, for ages from infancy to extreme old age, thus establishing normals that cannot be affected except as other groups equally large, and as carefully measured, might show that for other regions, races or social conditions other normals hold true. His statistics show a normal of 16.92 gm. hemoglobin percentage for men from twenty to sixty years, and 15.53 for women from twenty to eighty years. Since then Haden,[7] Osgood[8] and Wintrobe and Miller[9] have all found the hemoglobin for men from nineteen to thirty years to be about 15.8 gm., and Osgood[10] found an average of 13.7 gm. hemoglobin in 100 women of the same age group. As these averages for men and women from nineteen to thirty are from much large numbers than Williamson's, they are necessarily correct for the student class. In Chart 4 there are both of Williamson's normals for adults, and these later student averages are divided so that a given hemoglobin may be read as percentage of normal for the sex of a patient who is within the given age limits. The normal variation from average should be remembered. Osgood found it from 14 to 18 gm. for men (average 15.8), and from 12 to 15.5 (average 13.7) for young women, and Williamson states there is a 10 per cent variation from the average at birth, and 15 per cent in adult life.

CONSTANTS

Hemoglobin: 96 per cent globulin, 4 per cent hematin. Iron, 0.334 per cent, or 1 mg. iron per 300 mg. hemoglobin. Oxygen capacity, 1.34 c.c. per 1 gm. hemoglobin.

CONCLUSIONS

1. Normal hemoglobin means normal for age and sex, and should be determined by Williamson's or other standards, and so recorded.

2. The Dare hemoglobinometer should be discarded.

3. For clinical work hemoglobin may be estimated by the following methods: (a) Rough bedside estimations may be made by the Tallqvist or the Sahli. If the blood is below normal, a more accurate method should be used.

(b) The Newcomer disk, or the Newcomer hemoglobinometer as furnished by Bausch and Lomb is sufficiently accurate for clinical work. Blood dilutions made with their pipette can be carried to be read at leisure. When the color index is diagnostic, duplicate tests should be made, and an average of ten readings be taken from each dilution. If there is any doubt as to the accuracy of a disk, it should be checked by the van Slyke oxygen capacity method or an accurate ferrometric analysis through a wide range.

4. In the case of adults there is so long a period during which the normal hemoglobin scarcely varies that the percentage of variation may be added as a simple and vivid comparison, along with the grams per 100 c.c. blood. For infants and children the percentage of an arbitrary male standard, as used in the past, is only misleading. The hemoglobin observed should be recorded in grams, together with the normal for age and sex, which can be interpolated from Williamson's curve.[11]

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COLORIMETRIC ESTIMATION OF THE URINARY P_H*

By Victor C. Myers, and Edward Muntwyler, Cleveland, Ohio

AS ORDINARILY carried out the colorimetric estimation of the hydrogen-ion concentration of urine gives results which are too high by approximately 0.2 P_H, if one desires to know the P_H of the undiluted urine at body temperature. While it is true that an error† of 0.2 P_H in the estimation of the P_H of urine is of relatively little significance owing to the wide variations which may be encountered normally (4.8-8.0 P_H), still one should always know the accuracy and limitations of the method employed. In the past it has been assumed that a 10 or 25 fold dilution was without influence on the P_H of urine, and these dilutions are the ones which have been employed for urine. Hastings and Sendroy[1] have suggested a 1 to 5 dilution with water, while still more recently Myers and Muntwyler[2] have employed this dilution, but used as a diluent a saline solution because of its greater stabilizing effect on the dilution error. Employing water the error of dilution may be extremely variable. Diluting a urine of very high P_H where the carbonates form the major buffers will show a much greater dilution error than a highly concentrated urine of low P_H where phosphates form the buffers. By employing saline solutions with the reaction adjusted to suit each indicator range, this variable affect of water dilution is minimized and consequently makes all results comparable.

These same principles probably apply to fluid bacteriologic culture media as well as to urine, since one should have essentially the same temperature and dilution errors. Of course if a plus error as great as 0.3 P_H is of no consequence, then these errors need not be considered.

For their work Myers and Muntwyler[2] employed the bicolorimeter.[3] Owing to the fact that this instrument is not available in all laboratories it has seemed helpful to redescribe this method for use with the comparator box.

During the past five years we have employed the comparator box,† illustrated in Fig. 1, for class work and found it very satisfactory. As will be noted the cover and the bottom part of the box are in reality two comparator boxes and may be used as such when the urine is perfectly clear, and it is not necessary to employ a double row of tubes to obtain a satisfactory match. When not in use the cover is kept on the comparator box so that the buffer-indicator solutions will not be exposed to light.

*From the Department of Biochemistry, School of Medicine, Western Reserve University, Cleveland.

†Although the object of the present paper is to point out sources of error in the customary estimation of the urinary P_H and describe conditions which permit its estimation with an error of less than 0.1 P_H, still there are conditions under which this accuracy is unnecessary. The La Motte Chemical Products Company list a "Urinary P_H Outfit" in which, with the aid of a duplex indicator (combination of two indicators) the range P_H 4.4 to 8.0 is covered by 10 tubes differing by 0.4 P_H. This should be quite satisfactory for ordinary clinical work. They also make a similar comparator box containing 19 tubes differing by 0.2 P_H, which covers the P_H range 4.0 to 8.0.

†This special comparator box may be obtained from the La Motte Chemical Products Company, Baltimore, Md.

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The Clark-Lubs series of indicators are admirably adapted to the determination of the P_H of urine. The indicators bromcresol green (P_H 3.8 to 5.4), bromcresol purple* (P_H 5.2 to 6.8), bromthymol blue (P_H 6.0 to 7.6), and phenol red (P_H 6.8 to 8.4) nicely cover the range of hydrogen-ion concentration which may be encountered in urine. For most urines it will be found unnecessary to use the indicators bromcresol green and phenol red, although with urines which are strongly acid or those which are alkaline these two indicators may be necessary. Most urine specimens fall within the range of the indicator, bromcresol purple.

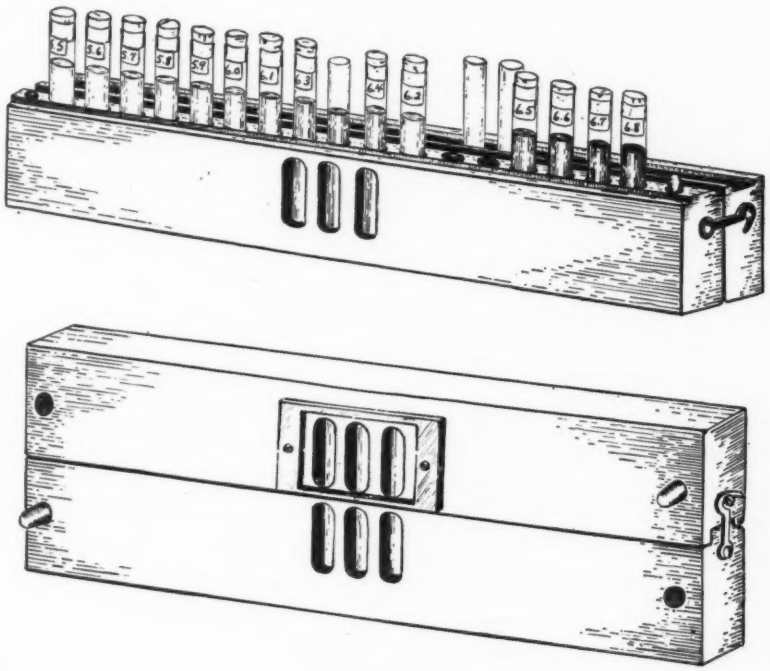

Fig. 1.

A series of Sørensen's M/15 molecular buffer phosphates are employed for the standards from P_H 5.2 to 8.6. For the range from 4.4 to 5.2 Clark's phthalate-NaOH standards are employed. The different standards are made up to differ by P_H 0.1, and it is therefore possible by interpolation to read to P_H 0.05.

INDICATOR DILUTING SOLUTIONS

The indicator diluting solutions are prepared as follows: To 780 c.c. of 0.9 per cent sodium chloride solution 100 c.c. of the desired diluted indicator solution are added. This, when diluted 4 to 5, gives the same concentration of dye as in the standard. Bromcresol purple, bromthymol blue, and bromcresol green in 0.04 per cent strength and phenol red in 0.02 per cent strength are prepared from 0.4 per cent stock solutions by dilutions of 1 to 10 or 1 to 20 with distilled water.

*Chlorphenol red covers the same P_H range as bromcresol purple and may be substituted for it. The former has the advantage that it does not show dichromatism and is more stable in the presence of strong alkali than bromcresol purple.

The stock solutions of the dye are prepared by grinding up one decigram with the molecular amount of alkali: 5.7 c.c. of 0.05 N NaOH for phenol red, 3.2 c.c. for bromthymol blue, 3.7 c.c. for bromcresol purple, and 3.2 c.c. for bromcresol green and diluting to 25 c.c. with water.

The saline-indicator diluting solutions are adjusted to a fairly definite P_H before use. The phenol red mixture is adjusted to about P_H 7.4 and the bromthymol blue to about P_H 7.2 by drawing CO_2 free air through the solution. The bromcresol purple and bromcresol green are adjusted to P_H 6.2 and 5.0 respectively by the addition of minute quantities of HCl. These solutions can best be kept in pyrex flasks or bottles.

TABLE I

COMPOSITION OF BUFFER MIXTURES

P_H	M/10 KH PHTHALATE*	M/10 NaOH	P_H	M/15 KH_2PO_4	M/15 Na_2HPO_4
	c.c.	c.c.		c.c.	c.c.
4.4	5	0.75	6.2	8.09	1.91
4.6	4	1.22	6.3	7.74	2.26
4.7	5	1.50	6.4	7.30	2.70
4.8	5	1.77	6.5	6.82	3.18
4.9	5	2.08	6.6	6.30	3.70
5.0	5	2.39	6.7	5.66	4.34
5.1	5	2.68	6.8	5.08	4.92
5.2	5	3.00	6.9	4.48	5.52
5.3	5	3.26	7.0	3.89	6.11
5.4	5	3.55	7.1	3.34	6.66
			7.2	2.80	7.20
	M/15 KH_2PO_4	M/15 Na_2HPO_4	7.3	2.32	7.68
			7.4	1.92	8.08
	c.c.	c.c.			
5.2	9.82	0.18	7.5	1.59	8.41
5.3	9.74	0.26	7.6	1.30	8.70
5.4	9.64	0.36	7.7	1.06	8.94
5.5	9.58	0.42	7.8	0.85	9.15
5.6	9.48	0.52	7.9	0.69	9.31
5.7	9.33	0.67	8.0	0.56	9.44
5.8	9.16	0.84	8.1	0.43	9.57
5.9	9.00	1.00	8.2	0.32	9.68
6.0	8.71	1.29	8.3	0.25	9.75
6.1	8.40	1.60	8.4	0.20	9.80

*The phthalate-NaOH mixtures are made up to a volume of 10 c.c.

The M/15 phosphate solutions are prepared from special reagent salts. The secondary sodium phosphate is prepared by dissolving 9.47 gm. of anhydrous Na_2HPO_4 or 11.88 gm. of the crystalline salt, $Na_2HPO_4.2H_2O$ in distilled water and making up to 1 liter, while the primary potassium phosphate is similarly prepared from 9.08 of KH_2PO_4.

The M/10 KH phthalate is prepared by dissolving 20.418 gm. of the salt in water and making up to 1 liter.

PREPARATION OF STANDARDS

For the P_H range 5.2 to 8.4 the buffer standards may most satisfactorily be prepared from Sørensen's phosphates, but if values more acid than P_H 5.2 are required other buffers must be employed. The Clark phthalate-NaOH solutions serve very well.*

*In our own work we have employed Merck's special reagent (Sørensen) primary and secondary phosphates. Buffer mixtures prepared from them checked satisfactorily with the electrometric method. Our acid potassium phthalate was obtained from the La Motte Chemical Products Co. The phosphate salts may also be obtained from them, but in the case of the secondary phosphate, they supply the crystalline salt, $Na_2HPO_4.2H_2O$.

If one does not care to prepare the buffer phosphate and phthalate solutions these may also be obtained from La Motte, as may individual sealed tubes covering the P_H range desired. These sealed tubes are said to be good for several years.

Ten (or 5) c.c. portions of the phosphate and phthalate-NaOH mixtures are prepared as given in Table I and 1.0 (or 0.5) c.c. of the corresponding indicator added. These solutions are then transferred to small pyrex test tubes employed in the comparator. To preserve them the tubes are tightly corked and paraffined.

COLLECTION OF URINE AND ESTIMATION OF P_H

To obtain accurate P_H values it is essential that the urine be perfectly fresh, and it may be necessary to collect the sample in a pyrex flask under oil with the aid of a long stem funnel to help avoid the loss of CO_2.

Measure 4 c.c. of the bromcresol purple saline mixture into the small pyrex tube of the comparator, add 1 c.c. of urine and mix by inverting several times. If special precautions for loss of CO_2 seem necessary (urines of high P_H), the determination may be made under oil, mixing with a small stirring rod. If the P_H of the specimen falls within the range of this indicator as it is likely to do, select the two standards which most nearly match the unknown by placing them on either side of the unknown in the comparator box. If the reading, for example, appears to fall between P_H 6.6 and 6.7 it may be taken as P_H 6.65. Since colorimetric readings carried out at room temperature are approximately 0.2 P_H higher than the true P_H at body temperature (38° C.), it is necessary to deduct 0.2 P_H to obtain the true P_H of the urine. In this case the corrected P_H would be P_H 6.45.

In case the P_H of the urine is outside the range of the bromcresol purple, the determination consists in repeating the determination employing the correct indicator system (e.g., if the urine is acid to bromcresol purple the bromcresol green system is employed; while if the urine is alkaline either the bromthymol blue or the phenol red systems are employed).

SUMMARY

A simple method for the colorimetric estimation of the P_H of urine (or fluid bacteriologic culture media) is described, which takes into account the errors incident to temperature, dilution, and salts.

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A SIMPLIFIED METHOD FOR WHITE BLOOD CELL COUNTS THAT IS APPLICABLE TO FIELD CONDITIONS*

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THE generally accepted methods of collecting blood for either the red or the white cell count are not practicable in the field unless the clinician carries the microscope to the patient. The difficulties attendant upon this procedure seriously limit the use of blood study as an aid in diagnosis.

Lewis and Shope[1] in studying blood from swine, suggest the use of potassium oxalate in undiluted blood to prevent clotting while the latter is being transported to the laboratory. I found that crystals of potassium oxalate appeared upon the counting chamber and interfered with the count. Blood which is treated with potassium oxalate must be diluted within a few hours to avoid spoilage by bacterial contamination.

The present study was undertaken in an effort to develop a technic whereby clinicians could conveniently collect samples and send them to a laboratory for the counts. Pigs' blood was used throughout the experiment.

An effort was made to dilute the blood immediately after bleeding and to eliminate the use of the standard diluting pipette.

Technic:

Nine and five-tenths c.c. of diluting fluid were measured accurately into 6-inch test tubes that were equipped with rubber stoppers and a few glass beads.

The animals were bled from one of the ear veins with an 18-gauge needle 3¼ inches in length. Approximately one cubic centimeter of blood was collected into a second test tube and by aid of a graduated 1 c.c. pipette exactly 0.5 c.c. of blood were measured into the tube containing the diluting fluid. This gave a dilution of one part of blood in nineteen parts of diluent. Care should be exercised to wipe the excess blood from the surface of the pipette.

In making the counts the tubes of diluted blood were vigorously shaken for exactly thirty seconds. The cover glass was placed upon the counting chamber and the diluted blood was transferred from the test tube to the counting chamber by means of a capillary pipette prepared from ordinary glass tubing. The blood was allowed to flow underneath the cover glass by capillary attraction. In an effort to check the technic of shaking the diluted blood, each half of the counting chamber was prepared and counted separately. The dilution tube was shaken exactly thirty seconds before transferring the blood to the counting chamber in each case.

All preparations were allowed to stand five minutes before making the counts. In making the count the technic recommended by Piney[2] was used.

Early in the work it was found that the diluting fluids recommended by Piney[3, 4] Mallory and Wright,[5] Cummer,[6] or Burnett,[7] which consisted of various dilutions of acetic acid tinged with carbo-gentian violet, were not adequate. If the counts were made within a few hours very little difficulty was experienced, but, if the counts were made some time after making the dilutions, the micro-

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scopic field was often clouded with débris and the cells were swollen, distorted or indistinct.

Twenty-four different preparations of various acids were tried. A description of the more promising results together with the outstanding failures are reported in Table I: Two stains were used in these tests. They consisted of a

TABLE I

SUMMARY OF RESULTS WITH VARIOUS DILUTING FLUIDS

CODE NUMBER	COMPOSITION OF DILUTING FLUID	AGE OF DILUTED BLOOD WHEN COUNTED	TEMPERATURE OF STORING DILUTED BLOOD	OBSERVATIONS IN COUNTING
1 A	2% acetic acid* faintly tinged with carbo-gentian violet	few hours	room temp.	The field was not clear, clumps of red cells and other débris interfered with the count.
3 A	2% acetic acid with 1% carbo-gentian violet	5 days	room temp.	Red cells faintly visible, white cells slightly brown, not swollen.
5 A	3% acetic acid with 0.5% carbo-gentian violet	few hours	room temp.	Clear field, white cells distinct.
		5 days	5° C.	Cells not clear, difficult to locate.
7 A	2% acetic acid with 1% methyl violet solution	few hours	room temp.	Cells slightly stained, quite distinct.
		3 days	room temp.	Cells slightly stained, quite distinct. Very little débris.
10 A	3% lactic acid† with 1% methyl violet solution	few hours	room temp.	Cells slightly stained, quite distinct, some débris.
		1 day	room temp.	Cells slightly stained, some débris.
11 A	2% citric acid with 1% methyl violet solution	few hours	room temp.	Cells slightly stained, quite distinct, some débris.
		1 day	room temp.	Cells overstained, very little débris.
12 A	2% oxalic acid with 1% methyl violet solution	few hours	room temp.	Cells a brownish tint, contrast to color of débris. Cells quite distinct.
		1 day	room temp.	Field covered with small and large particles. Cells yellowish brown.
13 A	4% oxalic acid with 1% carbo-gentian violet	few hours	room temp.	Cells sharply outlined, morphology distinct, stained lemon yellow, very little débris.
		1 day	5° C.	Cells a bright yellow, morphology quite distinct cells not distorted. A very fine débris over the field but does not interfere with count.
20 A	5% oxalic acid with 1% methyl violet solution	few hours	room temp.	Cells clear and distinct, stained a yellow color.
16 A	3% citric acid with 1% methyl violet solution	few hours	room temp.	Some débris of red cells, with cells stained faint lavender, cytoplasm clear but visible, nuclei stained.
		1 day	5° C.	Cells stained, but not clearly, much débris.

*The acetic acid used was Merck 36 per cent.
†The lactic acid was 75 per cent strength.

standard preparation of carbo-gentian violet[5] and a 1 per cent aqueous solution of methyl violet. When methyl violet was used in the diluting fluids, the amount was 1 per cent of the aqueous solution.

The most satisfactory preparations were 12 A and 20 A which consisted of 2 per cent and 5 per cent oxalic acid, respectively, and 1 per cent of a 1 per cent methyl violet solution. The yellow stained cells stood out in relief and were quickly and easily seen. The preparation 13 A, which contained 3 per cent

oxalic acid and 1 per cent carbo-gentian violet was almost as satisfactory. These three preparations were much superior to any of the others tried in the experiment.

Comparisons were made between the standard method of collecting blood samples using the hemacytometer, and the test tube method described in this article. Ten such comparisons were made by taking duplicate samples of blood from the same animal at the same time. The hemacytometers were filled directly from the hypodermic needles, while the blood for the tube tests was collected in separate test tubes. The average of the counts for these tests by the hemacytometer method was 13423 while the average by the test tube method was 12554.

An effort was then made to definitely check the error of both methods by making a series of counts from the same animal at the same bleeding. February 27, 1929, Pig No. 3055 was bled from the ear with a hypering needle. Five separate hemacytometers were filled one after the other. Five dilutions were then made from same needle into test tubes containing dilution fluid. The diluting fluid used was No. 20 A (5 per cent oxalic acid with 1 per cent of a 1 per cent aqueous solution of methyl violet). The dilution in each test was one in twenty.

The counts were made in duplicate using separate counting chambers. Each dilution was shaken exactly thirty seconds before transferring to the counting chamber. The cover glasses were placed upon the slides and the diluted blood was introduced from the edge. Two drops of diluted blood were discarded from each pipette. The third was wiped off with a clean cloth and the fourth was placed upon the slide. In each instance the preparation was allowed to stand five minutes before making the count.

TABLE III

HEMACYTOMETER METHOD

TUBE NO.	FIRST COUNT	SECOND COUNT	AVERAGE OF FIRST AND SECOND	DIFFERENCE OF FIRST AND SECOND
143	4960	3960	4460	1000
144	4200	4520	4360	320
145	5120	2920	4020	2300
146	5040	4480	4760	560
147	5960	5200	5580	760
Averages	5056	4216	4636	988

Range of difference by the hemacytometer method 3040

TABLE IV

TEST TUBE METHOD

TUBE NO.	FIRST COUNT	SECOND COUNT	AVERAGE OF FIRST AND SECOND	DIFFERENCE OF FIRST AND SECOND
148	4200	4640	4420	440
149	4480	4400	4440	80
150	4040	4560	4300	520
151	4600	4560	4580	40
152	4280	5080	4630	800
Averages	4212	4648	4470	376

Range of difference by the test tube method 1040

Table III records the counts by the hemacytometer method and Table IV records the counts by the test tube method. All of the counts represent the blood from the same animal at the same bleeding.

The average count by the hemacytometer method was 166 higher than by the test tube method. With the former method the range of differences was 2000 greater than by the test tube method.

The tubes numbered 148 to 152 inclusive were set aside at room temperature for five days.

On March 4 these tubes were recounted with the following results:

TUBE NO.	FIRST COUNT	SECOND COUNT	AVERAGE COUNT	DIFFERENCE BETWEEN TWO COUNTS
148	5080	4920	5000	160
149	4480	4400	4440	80
150	4600	4840	4720	240
151	4920	3920	4420	1000
152	3920	4560	4290	640
Averages	4600	4528	4574	424
		Range of differences 1160		

DISCUSSION

By making use of a previously measured tube of diluting fluid and a graduated pipette, dilutions of blood may be transported to the laboratory without danger of loss of the specimen. This is not possible when the standard diluting pipette is used. Subsequent to these experiments, a few tests were made in which the amount of diluting fluid was reduced to 1.9 c.c. and the amount of blood to 0.1 c.c. In these tests the blood was collected from a drop from a needle puncture or directly from the needle. The results indicate that the latter technic might well be substituted for the larger amounts of blood and diluting fluid.

When proper care is exercised in making the dilutions and in preparing the slide for the count, the results are more uniform when a pipette and a test tube are used in making the dilutions. The uniformity of count depends to a marked degree upon adequate shaking of the diluted blood. This technic enables the worker to transport the sample without danger. The count can be made at his convenience.

A more satisfactory preparation can be obtained by the use of 2 per cent to 5 per cent oxalic solution with 1 per cent of a 1 per cent methyl violet as a diluting fluid.

I wish to acknowledge the suggestion from Doctor S. H. Regenos to use a test tube in making the dilutions of blood.

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BLOOD SUGAR METHODS FROM A CLINICAL POINT OF VIEW*

By O. B. Pratt, M.D., and H. O. Swartout, B.A., M.S., Los Angeles, Calif.

SINCE 1920, when Folin and Wu[1] announced their improved procedure, now commonly known as the "standard Folin-Wu blood sugar method," many workers have tested it as to its chemical accuracy and clinical usefulness under varying conditions. Though it is probably still more widely used than any other blood sugar method, at times changes in technic have been proposed, and other methods have been suggested as superior to it. Most of the reports of work along these lines have appeared in the *Journal of Biological Chemistry*. Naturally, therefore, the chemical side of blood sugar methods has been emphasized more than the clinical.

During recent years the clinical importance of blood sugar tests has greatly increased, due largely to insulin therapy. A reasonably accurate idea of a patient's blood sugar level is often a prime point in diagnosis and a valuable guide in treatment. Biochemists are properly interested in the correct figure for average normal blood sugars, and in the question as to whether what appears to be blood sugar is really all sugar or partly one or more other compounds; but such matters are of less clinical moment. The clinician's purpose is adequately served, and the work of the clinical laboratory is made useful and dependable by any blood sugar method which, in the hands of the ordinary technician, gives a good check on the amount of deviation from normal in the case of both high and low sugars, no matter what the normal level for that particular method may be.

The work of several investigators who have attacked the blood sugar problem from the chemical side has clinical aspects which we believe deserve the review we aim to give them in this paper. The results of some of our own recent tests will also be considered, as they have a bearing on the subject. Of necessity, part of the discussion must concern itself with the chemistry of blood sugar methods; but more emphasis will be put upon those features which determine their clinical usefulness.

In 1923 Rothberg and Evans[2] announced the results of a series of studies of the standard Folin-Wu method. They found that it gave results too high for high sugars and too low for low sugars. In 1926 Oser and Karr[3] reported tests indicating similar errors, though they found the deviations somewhat less than those noted by Rothberg and Evans. Rockwood[4] confirmed the findings of Oser and Karr in this respect, and the results of our tests are in general agreement with theirs. Our figures indicate that, when using the single standard, a blood sugar at a level of about 200 mg. per 100 c.c. of blood will give results from 10 per cent to 12 per cent too high, while one near the 50 mg.

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level will turn out from 15 per cent to 18 per cent less than its true value—differences too great to be disregarded by the careful clinician, especially in the case of the low sugars.

Rothberg and Evans attributed these deviations to the abnormal behavior of colored colloidal solutions when diluted to different degrees. They advised the use of several different standards, together with a special type of tube which would permit of adjusting the dilutions, as a means of overcoming the difficulty. Our check tests only partly confirmed their conclusions. But, granting that their work was free from error, the added details of technic which they suggested would make the method of analysis unsatisfactorily complex for routine use.

For the purpose of calculating the proper values for blood sugars from observed colorimeter readings, Oser and Karr advised the use of a special table, corrected so as to offset the deviations indicated by their tests. This is a practical solution of the problem, and in our routine work we for a considerable time used such a table. Observation and inquiry, however, led us to the opinion that most laboratories employing the Folin-Wu method do not use corrected tables. As a consequence, much too low reports on low blood sugars must be very common.

Much of the blood sugar work during the past three or four years has been aimed at perfecting reagents or methods that would be specific for glucose as distinguished from other reducing substances in the blood. While of great chemical interest, not much of this work is an improvement on the standard Folin-Wu method from a clinical viewpoint. We may at once rule out as ill adapted for practical laboratory purposes all the methods which include yeast fermentation as a step in technic. These require the attention of a worker of more than average competence, as well as carefully controlled conditions, or else serious errors will often be made. Van Slyke and Hawkins'[5] gasometric method is cumbersome and time-consuming, compared with the Folin-Wu method. A new copper reagent proposed by Folin[6] early in 1926 is so weakly alkaline that all of the blood filtrates must for safety be neutralized to phenolphthalein before testing, a fact that argues against the routine use of this reagent. Furthermore, later in the same year, Folin and Svedberg,[7] in giving directions for handling the new copper reagent, brought to light another fact which makes it objectionable, it is not stable unless it is kept in full, tightly-stoppered bottles until shortly before using. Benedict's[8] 1928 copper reagent gives results that are believed to be very nearly correct, but it is also open to the criticism of instability and inconvenience. He advised that this reagent be kept as two separate solutions, which should be mixed not more than a day or two before being used. His[9] 1926 reagent is better, both as to convenience and keeping qualities.

At this point we digress briefly to note some interesting items connected with our own work. We have made hundreds of tests of the standard Folin-Wu method, as applied both to aqueous solutions of glucose and to blood filtrates with suitably adjusted glucose content, the tests covering the range corresponding to from 50 mg. to 400 mg. of glucose per 100 c.c. of blood. Our original purpose was to obtain data for a corrected table, such as proposed by Oser and

Karr. We give an abridged report of average results below, however, so that a rather surprising feature, noted after the tests were completed, may more easily be demonstrated.

ACTUAL CONCENTRATION OF GLUCOSE IN TEST SOLUTIONS	CALCULATED CONCENTRATION OF GLUCOSE BY FOLIN-WU TESTS	ACTUAL CONCENTRATION OF GLUCOSE IN TEST SOLUTIONS	CALCULATED CONCENTRATION OF GLUCOSE BY FOLIN-WU TESTS
mg.	mg.	mg.	mg.
50	42	100	100
60	54	120	123
70	65	140	147
80	77	160	170
90	89	180	195

While studying our results it occurred to us that if a quantity of color equivalent to 15 mg. or 16 mg. of glucose per 100 c.c. of blood could be added to each tube, standard included, the whole series would be brought so nearly into line that no result would differ much from what it ought to be. For instance, the tube which, compared with a standard of 100 mg. per 100 c.c. gave a reading that calculated out at 42 mg. per 100 c.c. should have given 50 mg. per 100 c.c., that which led to a report of 170 mg. per 100 c.c. should have given 160 mg. per 100 c.c., etc. Now the ratio of $(42 + 15) : (100 + 15)$ nearly equals $50 : 100$, $(42 + 16) : (100 + 16)$ equals $50 : 100$, $(77 + 15) : (100 + 15)$ equals $80 : 100$, $(77 + 16) : (100 + 16)$ nearly equals $80 : 100$, $(170 + 15) : (100 + 15)$ nearly equals $160 : 100$, $(170 + 16) : (100 + 16)$ nearly equals $160 : 100$, and so on through the list.

The above figures were derived from tests made with single standards, but we found the same mathematical relations to hold in the case of results obtained by using more concentrated test solutions read against double standards. We make no pretense of knowing all the reasons for this peculiarity. It may be that the deviation of colored colloidal solutions from Beer's law[20] partly explains it. Perhaps something in the copper reagent affects the glucose in such a way as to prevent a fairly constant quantity of it in each tube from entering into the reduction reaction. The fact that some tests which we made on solutions representing 20 mg. of glucose per 100 c.c. of blood gave almost no color at all points to this possibility. But no matter what the explanation may be, it is evident that if the copper reagent of Folin and Wu could be modified so as to aid in the production of added color to the extent called for by our figures, their method would give proportionately accurate results for both high and low sugars.

With this thought in mind we set out to alter the Folin-Wu copper reagent in the desired direction, meanwhile reinvestigating such other reagents as were not clinically undesirable from other points of view. We had already met with an encouraging measure of success when we began testing Benedict's 1926 copper and acid reagents. These gave us results so nearly correct that we went no further with our work in modifying the Folin-Wu reagent, feeling that it would serve no good purpose to develop a new reagent that would be no improvement over one already proposed.

According to our tests, these Benedict reagents, used with standard Folin-Wu filtrates, lead to reports on blood sugars as low as 40 mg. per 100 c.c. that in proportion to each other and to the standards employed are accurate within the limits of observational error. On high sugars the error may run as high as 2 per cent or 3 per cent, the reports being too low to this extent. Such a deviation in the case of a high sugar is clinically insignificant, but if desired it can easily be avoided by using a smaller quantity of filtrate for the test.

It may be of interest to mention that when testing Benedict's 1926 reagents we did not find the blanks negligible. Since, however, the slight color due to the blanks affects the standards as well as all of the test solutions, we have here a possible partial explanation of the closer agreement between actual and theoretical sugar values which these reagents give as compared with the standard reagents of Folin and Wu. It is more color in all the tubes which is needed to bring the Folin-Wu results into line, and the blanks of Benedict's reagents supply a little additional color.

We have found Benedict's 1926 copper reagent to have enough alkalinity to offset the acidity of filtrates from blood samples that have been treated with many times the recommended quantities of any of the common anticoagulants, comparing favorably with the standard Folin-Wu reagent in this respect. This is a valuable feature in practical work, for the average clinical laboratory must often deal with blood samples which have been treated with a considerable excess of the compounds added to prevent clotting.

CONCLUSION

While Benedict's 1928 copper reagent gives results that are probably nearer the correct levels for blood sugars, his 1926 reagent is preferable from the standpoints of stability and convenience. Though this reagent may lead to results that are slightly too high all along the line, these results are consistent among themselves and closely proportional to the standards. Our tests indicate that Folin-Wu blood filtrates used with Benedict's 1926 copper and acid reagents, made according to directions given in the *Journal of Biological Chemistry*,[11] provide a blood sugar method that is suited to the ordinary clinical laboratory and that will give results upon which the clinician can safely base his diagnoses and treatments. We know of one large hospital that is using this combined method at present. There may be others. We feel that we are justified in adopting it as a routine procedure in our own laboratory.

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A CHART FOR ADJUSTING THE DIET IN DIABETES*

By Curtis Brüen, M.D., New York City

NITROGENOUS equilibrium, caloric requirement, ketogenic-antiketogenic balance, and glucose equivalent tolerance together determine the diabetic diet. The wear and tear quota fixes the protein minimum. The caloric requirement is satisfied according to the equation,

$$C = 4.1CH + 4.1P + 9.3F \quad (1)$$

Ketogenic-antiketogenic balance is maintained by the relationships of the equation,

$$F = 2CH + 0.546P \quad (2)^1$$

Tolerance sets a limit within which the glucose equivalent must fall unless insulin be given.

Charts are available for reading off food mixtures in ketogenic-antiketogenic balance. The alignment chart of Wilder based on a special gram molecular calculation indicates the grams of carbohydrate and fat required to meet the caloric requirement with a given protein allowance without clinically significant accumulation of acetone bodies.[2] Given the total calories and the percentage of the total calories to be furnished by protein, the complex line diagram of Hannon and McCann derived from the above equations[3] indicates the grams of protein, the grams of carbohydrate, and the factor by which the grams of carbohydrate is multiplied to give the grams of fat.[4] A single diet follows from each set of conditions.

Graphs of simultaneous equations permit of more flexibility. A system of graphs with serial protein values from a near-minimal wear and tear quota[5] to an arbitrary upper limit is drawn for equation (2). The segments above their intersections with the individual graphs of this system are drawn for systems of graphs for equation (1) having corresponding protein values but serial values of total calories. The widest range of practicable values of the several foodstuffs which satisfy the equations individually and simultaneously is provided.

Chart I. Diabetic Diet.—Directions: (1) Identify the graph for the required number of grams of protein; (2) run along it to the point where a member of the bank of graphs for the required number of calories rises from it; (3) read off the coordinates of this point for the number of grams of carbohydrate and fat in the threshold diet; (4) read off the coordinates of points higher up on this graph for diets of increasing carbohydrate; (5) read off the coordinates of points along its imaginary projection beyond the intersection for diets of impending ketosis.

A suitable combination is selected from among the serial values of protein and of calories available. The indicated graph of caloric equivalent mix-

*Received for publication, November 10, 1929.

tures of carbohydrate and fat satisfies these conditions throughout its length. Its point of simultaneity with the graph of ketogenic-antiketogenic balance satisfies in addition the relationships of the threshold of ketosis. Values within this limit are more remote from the development of ketosis but require more of the capacity to metabolize glucose. Values beyond it require less of

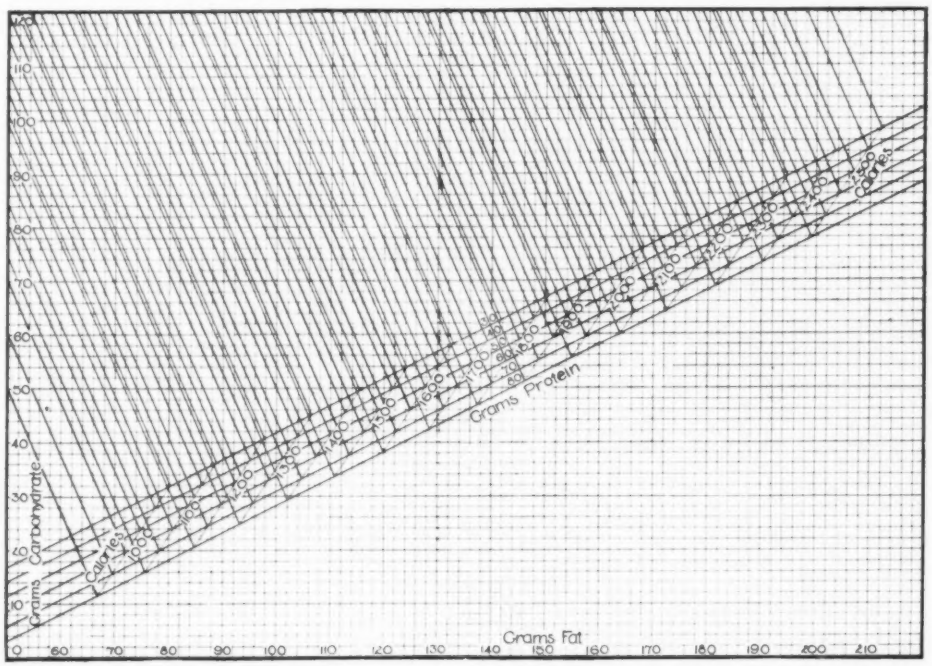

Chart 1.

the capacity to metabolize glucose but are less proof against the development of ketosis. The diet can be maintained at once along physiologic lines and according to the clinical indications.

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A GLASS LIGHT FILTER FOR FOLIN'S NEW MICRO BLOOD SUGAR METHOD*

By Henry Tauber, Ph.D., Brooklyn, N. Y.

FOLIN, in one of his later papers,[1] suggests the use of a colored filter paper or a special lamp as a light filter to get uniformity in reading because of the disturbing effect of the excess of the yellow potassium ferricyanide solution.

We have cooperated with the Klett Manufacturing Co. in the preparation of a glass light color filter which is a yellow glass disc, 15 mm. in diameter, which should be placed on top of the ocular of the colorimeter.

TEST OF THE GLASS LIGHT COLOR FILTER

The glass color filter is placed on the ocular of the colorimeter. One cup is filled half with a 0.2 per cent potassium ferricyanide solution and the other cup is filled half with water. Both plungers are set at 20 mm. and, if zero point and light are equal, the two fields look uniformly yellow.

Table I shows comparative figures of Folin's micro method with the glass disc color filter and the original Folin-Wu method. The bloods are those of diabetic patients.

TABLE I

SAMPLE NO.	SUGAR IN MG. PER CENT		
	FOLIN-WU	FOLIN	DIFFERENCE
1	222	220	2
2	160	148	12
3	320	312	8
4	142	135	7
5	266	252	14
6	400	388	12
7	166	156	10
8	160	153	7
9	117	111	6
10	306	290	16
11	250	228	22
12	220	217	3
13	152	147	5
14	302	290	12
15	92	86	6
16	105	100	5

This corroborates Folin's[2] lower findings with the micro method.

Table II shows figures of glucose solutions of various concentrations.

It is important to note that the lower findings of the micro method as compared to Folin-Wu's original method are due to the method itself as seen by the fact that the figures in both the glucose solutions and blood sugars are lower.

*From the Department of Laboratories of Beth Moses Hospital, Brooklyn, N. Y.
Received for publication, November 22, 1929.

TABLE II

SOLUTION NO.	MG. PER CENT GLUCOSE	FOLIN	DIFFERENCE
1	150	148	2
2	200	196	4
3	250	235	15
4	300	290	10
5	350	345	5
6	400	392	8
7	450	445	5
8	500	482	18
9	550	533	17
10	600	590	10
11	700	660	40

For the precipitation of the blood proteins in Folin-Wu's method, we used a precipitation mixture consisting of 7 parts of distilled water, 1 part of a 10 per cent sodium tungstate solution, and 1 part of 2/3 N sulphuric acid. To 9 parts of this mixture, in an Erlenmayer flask, 1 part of blood is added, drop by drop, while rotating the flask, and filtered after a few minutes.

We have made a great number of blood-sugar determinations using this mixture, which we compared with the original method of precipitation as used by Folin and Wu, and found that both results agree. Occasionally, a precipitate is formed in the mixture which has no effect on the blood sugar values.

The advantages of the glass disc as compared to Folin's filter paper light screen or special lamp are:

1. The disc saves time in routine examination.

2. The filter light papers tend to curl up due to the heat of the colorimeter lamp.

3. The disc is inexpensive and can be used for any plunger.

The author is indebted to Mr. Klett and Mr. Daniel for their assistance in preparing the disc.

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1000 PRECIPITATION TESTS FOR SYPHILIS WITH SMALL QUANTITIES OF DEFIBRINATED FINGER BLOOD (CLINICAL AND SEROLOGIC COMPARISON)*

By B. S. Kline, M.D., and Benjamin Levine, M.D., Cleveland, Ohio

THE simple precipitation test for syphilis with small quantities of defibrinated finger blood described previously[1] has been found in 1000 tests to be more sensitive than the serum Wassermann test with the same antigen and almost as sensitive as the serum microscopic slide precipitation test. No false positive reactions occurred in any of the tests in this series. In 121 cases, the finger blood test performed with the slight changes noted below gave results agreeing with the clinical condition of the patients as often as those of the serum microscopic slide precipitation test and more frequently than those of the serum Wassermann test.

The finger blood precipitation test for syphilis is satisfactory for use in the diagnosis of syphilis in all cases and since it requires but a small quantity of comparatively easily obtainable blood, it is particularly useful in the diagnosis of syphilis in infants and children. Furthermore, after satisfactory blood typing and matching, the finger blood test for syphilis, since it is reliable and takes but about eight minutes to do, is an excellent method for determining the suitability of blood donors immediately before transfusion.

For the study presented below, blood was obtained from the finger and defibrinated for the finger blood test. At the same time a larger quantity was drawn from an arm vein to furnish serum for the microscopic slide precipitation test and for the Wassermann test. Over 60 per cent of the blood specimens were obtained from syphilitic patients. Of these over 80 per cent showed ± to ++++ reactions in one or more tests.

The clinical and serologic comparison of the finger blood test with other tests for syphilis is given in Tables I to IV.

Table I shows the close agreement of the finger blood test with the serum slide precipitation test. There is less agreement of the finger blood test with the Wassermann test.

Table II shows the results of the finger blood test to be more sensitive than those of the Wassermann test and almost as sensitive as those of the slide precipitation test.

Table III shows the false negative reactions in the various stages of syphilis. The very sensitive finger blood test gave one and a half times as many false negative reactions as the very sensitive microscopic slide precipitation test, whereas the very sensitive Wassermann test gave over two and one-half times as many.

*From the Laboratory Department and the Department of Syphilology of the Outpatient Clinic, Mount Sinai, Hospital.

Received for publication, November 2, 1929.

TABLE I

SEROLOGIC COMPARISON OF FINGER BLOOD PRECIPITATION TEST WITH HEATED SERUM TESTS FOR SYPHILIS WITH THE SAME ANTIGEN

	FINGER BLOOD TEST AND SERUM SLIDE PRECIPITATION TEST					FINGER BLOOD TEST AND WASSERMANN TEST*				
	AGREEMENT	RELATIVE AGREEMENT	AGREEMENT AND RELATIVE AGREEMENT	DISAGREEMENT	TOTAL TESTS	AGREEMENT	RELATIVE AGREEMENT	AGREEMENT AND RELATIVE AGREEMENT	DISAGREEMENT	TOTAL TESTS
Very sensitive antigen emulsion	619 Tests 80.28%	141 Tests 18.29%	760 Tests 98.57%	11 Tests[1] 1.43%	771	551 Tests 75.07%	130 Tests 17.71%	681 Tests 92.78%	53 Tests[3] 7.22%	734
Sensitive antigen emulsion	806 Tests 80.6%	180 Tests 18.0%	986 Tests 98.6%	14 Tests[2] 1.4%	1000	630 Tests 72.58%	167 Tests 19.24%	797 Tests 91.82%	71 Tests[4] 8.18%	868

[1] 11 Disagreements:
- 10 Negative finger blood tests, positive serum slide tests.
- 1 Positive finger blood test, negative serum slide test.

[2] 14 Disagreements:
- 13 Negative finger blood tests, positive serum slide tests.
- 1 Positive finger blood test, negative serum slide test.

[3] 53 Disagreements:
- 24 Positive finger blood tests, negative Wassermann tests.
- 29 Negative finger blood tests, positive Wassermann tests.

[4] 71 Disagreements:
- 50 Positive finger blood tests, negative Wassermann tests.
- 21 Negative finger blood tests, positive Wassermann tests.

*Wassermann Test Cleveland Method.
(Very sensitive antigen containing 0.3 per cent cholesterin, Sensitive antigen containing 0.06 per cent cholesterin.)

TABLE II

CLINICAL COMPARISON OF FINGER BLOOD TEST AND SERUM TESTS FOR SYPHILIS WITH THE SAME ANTIGEN

	FINGER BLOOD PRECIPITATION TEST				HEATED SERUM SLIDE PRECIPITATION TEST				HEATED SERUM WASSERMANN TEST			
	VERY SENSITIVE EMULSION		SENSITIVE EMULSION		VERY SENSITIVE EMULSION		SENSITIVE EMULSION		VERY SENSITIVE EMULSION		SENSITIVE EMULSION	
	AGREEMENT	DISAGREEMENT	AGREEMENT	DISAGREEMENT	AGREEMENT	DISAGREEMENT	AGREEMENT	DISAGREEMENT	AGREEMENT	DISAGREEMENT	AGREEMENT	DISAGREEMENT
Syphilitic sera this series ± to ++++ in one or more tests	370 87.47%	53 12.53%	397 76.79%	120 23.21%	387 91.49%	36 8.51%	454 87.81%	63 12.19%	319 77.80%	91 22.20%	256 55.29%	207 44.71%
Syphilitic sera this series negative to ++++	445 89.36%	53 10.64%	501 80.68%	120 19.32%	462 92.77%	36 7.23%	558 89.86%	63 10.14%	394 81.24%	91 18.76%	350 62.84%	207 37.16%
Syphilitic and nonsyphilitic sera this series (over 50% positive syphilitic sera)	716 93.11%	53 6.89%	871* 87.89%	120† 12.11%	723 95.26%	36 4.74%	928 93.64%	63 6.36%	650 87.72%	91 12.28%	674 76.50%	207 23.50%
Approximate result if series contained 20% positive syphilitic sera (high average)	Approx. 2062 97.49%	53 2.51%	Approx. 2465 95.36%	120 4.64%	Approx. 2079 98.30%	36 1.70%	Approx. 2522 97.56%	63 2.44%	Approx. 1959 95.56%	91 4.44%	Approx. 2108 91.06%	207 8.94%

*871, †120, and 9 = 1000 total tests.

Evaluation:
Negative in all tests with syphilitic sera or non-syphilitic sera = Agreement.
± to ++++ in all tests with syphilitic sera = Agreement.
Negative in one test, ± to ++++ in one or both other tests with syphilitic sera = Disagreement.

9 Clinically doubtful cases as follows:

	FINGER BLOOD TEST		SERUM PRECIPITATION TEST		WASSERMANN TEST	
	SENSITIVE	VERY SENSITIVE	SENSITIVE	VERY SENSITIVE	SENSITIVE	VERY SENSITIVE
++		1				
± or +	4	4	2	2		
Negative	5		7	3	9	5
Total cases	9	5	9	5	9	5

TABLE III

CLINICAL COMPARISON OF TESTS FOR SYPHILIS ONE OR MORE OF WHICH GAVE A ± TO ++++ REACTION WITH ANALYSIS OF DISAGREEMENTS

	TOTAL TESTS	AGREEMENT	DISAGREEMENT	DISAGREEMENTS, FALSE NEGATIVES, SYPHILIS				
				PRIMARY	SECONDARY	TERTIARY	CENTRAL NERVOUS SYSTEM	CONGENITAL
Very sensitive finger blood test	423	370 87.47%	53 12.53%	8 1.89%	30 7.09%	7 1.65%	4 0.95%	4 0.95%
Very sensitive slide test	423	387 91.49%	36 8.51%	10 2.36%	16 3.78%	7 1.65%	1 0.24%	2 0.47%
Very sensitive Wassermann test	410	319 77.80%	91 22.20%	14 3.41%	38 9.27%	34 8.29%	1 0.24%	4 0.98%
Sensitive finger blood test	517	397 76.79%	120 23.21%	22 4.26%	66 12.77%	22 4.26%	5 0.97%	5 0.97%
Sensitive slide test	517	454 87.81%	63 12.19%	11 2.13%	34 6.58%	15 2.90%	1 0.19%	2 0.39%
Sensitive Wassermann test	463	256 55.29%	207 44.71%	22 4.75%	111 23.97%	61 13.17%	6 1.30%	7 1.51%

Table IV shows the results of the finger blood test in 121 cases, by the technic described below, to be as sensitive as those of the slide precipitation test and more sensitive than those of the Wassermann test.

DISCUSSION OF THE TECHNIC OF THE FINGER BLOOD PRECIPITATION TEST FOR SYPHILIS

Many of the finger blood tests reported above were done as outlined in a previous report.[1] In addition, many were done with antigen emulsions of different cholesterin content with, consequently, different optimum quantities of salt solution. Furthermore, it was found that the addition of a small quantity of acetic acid to the sensitive antigen emulsion increased its sensitivity equal to that of heating it at 50° C. for twenty minutes. It was also found that more cholesterin could be used in the antigen emulsions with advantage. A most important factor affecting the results was found to be the hydrogen-ion concentration of the distilled water used in the preparation of the antigen emulsions and the salt solution. It was also found better to lake the blood with 2 c.c. instead of 1½ c.c. of distilled water and, therefore, to increase the size of the outer chambers on the slides.

The details of the test considered most satisfactory of those done in this study follow:

FINGER BLOOD PRECIPATION TEST FOR SYPHILIS

1. Pipette 0.04 c.c. of defibrinated finger blood into each inner chamber on the 3 by 2 inch glass slide.

2. Allow one drop (about 0.015 c.c.) of 4 per cent sodium chloride solution to fall from a capillary pipette into each inner chamber containing blood and rotate the slide quite vigorously for one minute.

3. Allow two small drops (each about 0.007 c.c.) of sensitive antigen emulsion to fall from a capillary pipette into the first blood salt solution mixture. In the same manner allow a similar quantity of very sensitive antigen emulsion

TABLE IV

CLINICAL COMPARISON OF FINGER BLOOD TEST BY TECHNIC DESCRIBED IN THIS PAPER AND SERUM TESTS FOR SYPHILIS WITH THE SAME ANTIGEN

	FINGER BLOOD TEST (TECHNIC DESCRIBED IN THIS PAPER)				HEATED SERUM SLIDE PRECIPITATION TEST				HEATED SERUM WASSERMANN TEST			
	VERY SENSITIVE EMULSION		SENSITIVE EMULSION		VERY SENSITIVE EMULSION		SENSITIVE EMULSION		VERY SENSITIVE EMULSION		SENSITIVE EMULSION	
	AGREEMENT	DISAGREEMENT	AGREEMENT	DISAGREEMENT	AGREEMENT	DISAGREEMENT	AGREEMENT	DISAGREEMENT	AGREEMENT	DISAGREEMENT	AGREEMENT	DISAGREEMENT
Syphilitic sera this series ± to ++++ in one or more tests	75 92.59%	6 7.41%	72 88.89%	9 11.11%	75 92.59%	6 7.41%	76 93.83%	5 6.17%	61 77.22%	18 22.78%	44 56.41%	34 43.59%
Syphilitic sera this series negative to ++++	85 93.41%	6 6.59%	82 90.11%	9 9.89%	85 93.41%	6 6.59%	86 94.51%	5 5.49%	71 79.78%	18 20.22%	54 61.36%	34 38.64%
Syphilitic and nonsyphilitic sera this series (over 65% positive syphilitic sera)	115* 95.04%	6† 4.96%	112 92.56%	9 7.44%	115 95.04%	6 4.96%	116 95.87%	5 4.13%	101 84.87%	18 15.13%	83 70.94%	34 29.06%
Approximate result if series contained 20% positive syphilitic sera (high average)	Approx. 399 98.52%	6 1.48%	Approx. 396 97.78%	9 2.22%	Approx. 399 98.52%	6 1.48%	Approx. 400 98.77%	5 1.23%	Approx. 377 95.44%	18 4.56%	Approx. 356 91.28%	34 8.72%

*115, †6, and 2 in clinically doubtful cases = 123 total tests.

Evaluation:

Negative in all tests with syphilitic sera or nonsyphilitic sera = Agreement.
± to ++++ in all tests with syphilitic sera = Agreement.
Negative in one test, ± to ++++ in one or both other tests with syphilitic sera = Disagreement.

to fall into the second blood salt solution mixture. Rotate the slide with moderate vigor for four minutes.

4. Allow 2 c.c. of distilled water to fall from a 25 c.c. pipette (graduated in tenths) into each mixture (the mixtures spill over to the outer rings) and rotate the slide very gently for one minute to completely lake the blood.

5. Read the results at once or at any time for fifteen minutes after laking is complete, through the microscope at a magnification of about 100 times (low power 16 mm. objective, eyepiece 10X or 12½X) and record in terms of pluses according to the degree of clumping and the size of the clumps.

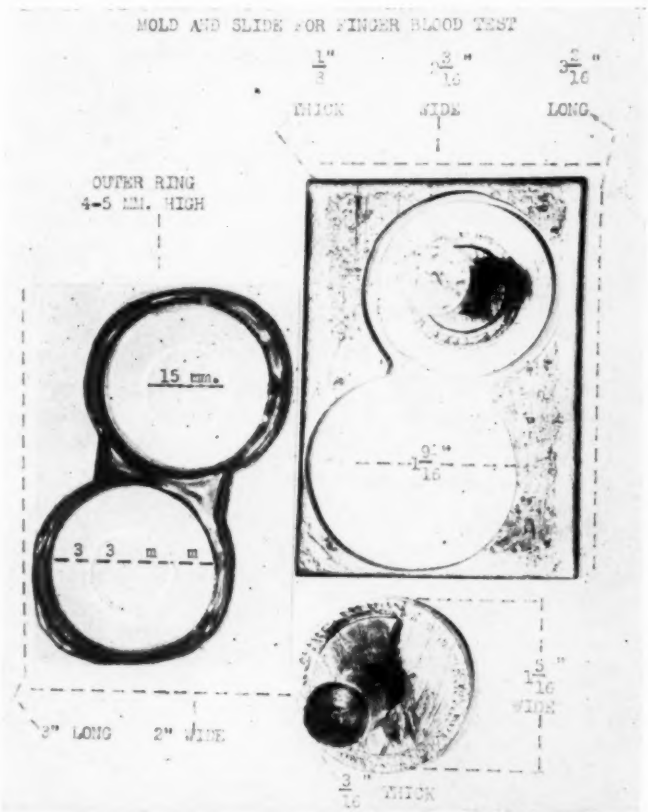

FIG. 1.

Six sensitive and six very sensitive tests may be done at one time with proper holders.

MATERIALS FOR THE FINGER BLOOD PRECIPITATION TEST FOR SYPHILIS

These are described in detail and illustrated in the first report.[1] Further experience with the test has led to the use of more distilled water in laking the blood. Accordingly, slides with larger wax outer rings than previously described are used. (See illustration of mold and slide.)

The pipette for the 4 per cent salt solution is a capillary pipette made from

glass tubing 8 to 10 mm. in diameter with capillary tube about ¾ mm. in diameter delivering a drop (by gravity) about 0.015 c.c. (33 drops per ½ c.c.).

The pipette for the antigen emulsions are similar pipettes with tubes about ⅖ mm. in diameter delivering drops (by gravity) about 0.007 c.c. (71 drops per ½ c.c.).

Four per cent salt solution. This is made with sodium chloride (c. p. or Reagent Merck) and distilled water of P_H 5.4 to 6.0. The P_H of the distilled water is determined simply by adding to 0.25 c.c. of it a drop of chlorphenol red indicator (La Motte). Water satisfactory for use gives a light to medium reddish-purple color. Water of P_H 5.2 or less gives a yellow color with the indicator and is not as satisfactory.

Antigen: The antigen is a lipid obtained by precipitation in acetone at 50° C. to 37° C. of concentrated absolute alcohol extract of beef heart muscle powder (Difco). The details of its preparation are given in a previous report.[2]

Antigen emulsions containing more cholesterin than formerly employed have been found more satisfactory for the finger blood test. Likewise it has been found more convenient to increase the sensitivity of the antigen emulsion by the addition of a small quantity of acetic acid than to heat the emulsion at 50° C. for twenty minutes as previously. The antigen emulsions considered best for the finger blood test are as follows:

Sensitive Antigen Emulsion

0.85 c.c. Distilled water (P_H 5.4 to 6.0).
1.25 c.c. of 1 per cent cholesterin (Pfanstiehl C. P.) in absolute ethyl alcohol (99+ per cent). (Prepared in about forty-five minutes by placing in an oven at 50° to 56° C. and shaking gently a few minutes at fifteen minute intervals.)
0.1 c.c. Antigen.
2.2 c.c. of 0.85 per cent sodium chloride (c. p. or Reagent, Merck) solution (made with distilled water P_H 5.4 to 6.0).

Very Sensitive Antigen Emulsion

2.0 c.c. of sensitive finger blood test antigen emulsion.
0.2 c.c. ½ per cent glacial acetic acid. (Distilled water P_H 5.4 to 6.0.)

The sensitive antigen emulsion for the finger blood test has the same formula as the very sensitive antigen emulsion for the microscopic slide precipitation test with serum and like it is prepared as follows:

Into a one-ounce bottle, 0.85 c.c. of distilled water is pipetted.

The bottle is held at an angle and the 1 per cent cholesterin in absolute ethyl alcohol (99+ per cent) is allowed to run along the side of the neck of the bottle.

The bottle is gently rotated from the neck for twenty seconds.

The bottle is held at an angle again and 0.1 c.c. of antigen is pipetted against the side of the neck from a 0.2 c.c. pipette (graduated in thousandths).

The bottle is promptly stoppered with a cork and shaken fairly vigorously (the fluid thrown from bottom to cork and back) for one minute.

Lastly, the 0.85 per cent sodium chloride solution is allowed to run in quite rapidly, the bottle is stoppered again and shaken as previously for one minute.

The emulsions, when examined through the microscope, at a magnification of about 100 times, show numerous very fine particles but no clumps whatever.

One-half hour after the preparation of the sensitive finger blood test emulsion, 2 c.c. of it is pipetted into a one-ounce bottle.

Two-tenths c.c. of ½ per cent glacial acetic acid is then pipetted against the side of the neck of the bottle. The bottle is stoppered well with a cork and shaken quite vigorously as before for one minute. The acidified emulsion is the very sensitive finger blood test emulsion.

The emulsions are thoroughly satisfactory for use for six hours after their preparation. After this time there is a slight steady decline in their antigenic power.

The defibrinated finger blood keeps well in the humidor in the refrigerator for at least twenty-four hours. Sixty-eight specimens kept in the refrigerator in sealed glass tubes made from 3 mm. glass tubing (about 2 mm. inside diameter) gave results, as late as six weeks later, differing but little from those with the blood when first obtained.

CONCLUSIONS

1. The simple precipitation test for syphilis with small quantities of defibrinated finger blood described previously has been found in 1000 tests to be more sensitive than the serum Wassermann test with the same antigen and almost as sensitive as the serum microscopic slide precipitation test. No false positive reactions occurred in any of the tests in this series. In 121 cases, the finger blood test performed with the slight changes noted above, gave results agreeing with the clinical condition of the patients as often as those of the serum microscopic slide precipitation test and more frequently than those of the serum Wassermann test.

2. The finger blood precipitation test for syphilis is satisfactory for use in the diagnosis of syphilis in all cases, and since it requires but a small quantity of comparatively easily obtainable blood, it is particularly useful in the diagnosis of syphilis in infants and children. Furthermore, after satisfactory blood typing and matching, the finger blood test for syphilis, since it is reliable and takes but about eight minutes to do, is an excellent method for determining the suitability of blood donors immediately before transfusion.

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MODIFICATIONS IN THE METHOD FOR THE DETERMINATION OF CHOLESTEROL IN BLOOD*

By S. L. Leiboff, A.M., New York City

IN 1924 the author[1] simplified the method for determination of cholesterol in blood by absorbing the blood on filter paper and extracting the cholesterol from the filter paper with chloroform. A special extraction tube was

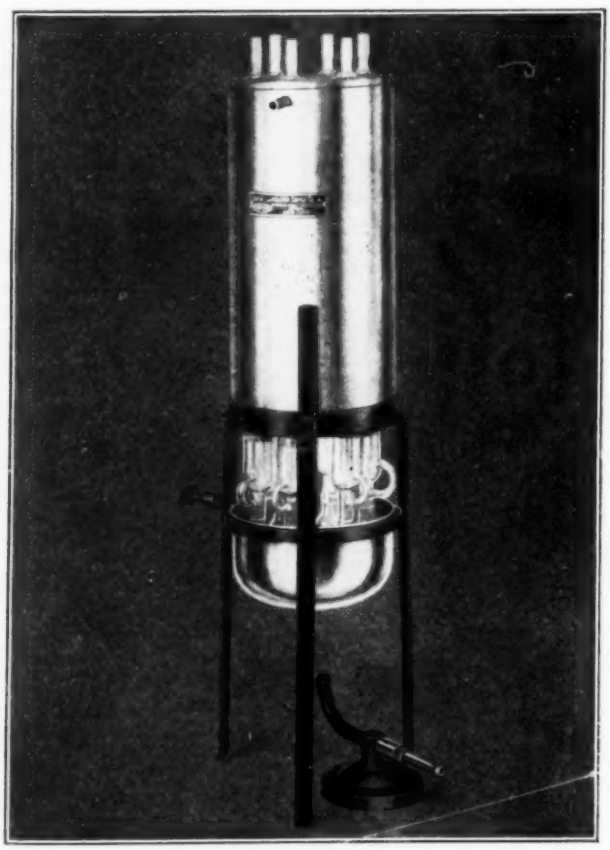

Fig. 1.

devised[2] in which the whole operation was performed without transferring material thereby avoiding possible loss. For the extraction a multiple condenser[3] was used. In this condenser six samples of blood could be extracted simultaneously. The condenser is of very simple construction and occupies a small space in the laboratory.

*From the Biochemical Laboratory of Lebanon Hospital, New York.
Received for publication, December 19, 1929.

A number of improvements have now been made in the apparatus which make the extraction even more accurate and allow the easier handling of the apparatus.

The most important change was made in the condensing tube C (Fig. 2). Originally this tube was constricted at the bottom; the diameter of this constricted portion was much narrower than the rest of the tube. This was found to be faulty in that it did not always allow the free passage of chloroform, thus causing some of the chloroform to collect in the condensing tube above the constricted portion. Since the constricted parts of the different condensing tubes differed somewhat in diameter no uniform extraction per unit of time was possible with different condensing tubes, since the amount of extraction depends upon the number of drops of chloroform passing through the filter paper.

This difficulty was entirely and satisfactorily overcome by eliminating

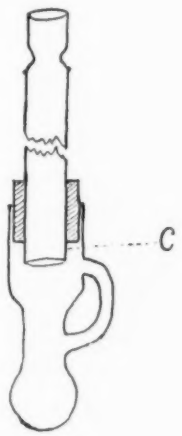

Fig. 2.

the constricted portion, the whole tube being of the same diameter. A large number of these improved condensing tubes were tested as to the number of drops passed per unit interval of time and very uniform results were obtained. Also a large number of bloods were extracted and cholesterol determinations performed in triplicates; very good checks were obtained. After extraction of the filter papers for half an hour, no more cholesterol could be extracted, thus showing that the first extraction was complete.

Another improvement was made in the substitution of a small copper water-bath for the large glass beaker. By using a beaker it was necessary to raise the whole apparatus when extraction tubes had to be placed in or removed from the water-bath. This is no longer necessary since the copper water-bath is held by an iron ring which may be raised or lowered by means of a clamp attached to one of the legs of the tripod, as shown in Fig. 1. The low form gas burner was found more suitable than the electric stove, though the electric stove may be used.

In preparing the blood for extraction it is of utmost importance to use the right grade of filter paper.* An extraction paper to be efficient should be very porous and not too hard; under such conditions the blood is exposed to a large number of pores in the paper and is easily absorbed. The large pores also allow the free passage of the chloroform, thus producing efficient extraction. When a filter paper is too hard its porosity is greatly diminished and a greater resistance is offered to the passage of the extracting fluid; as a result some drops of fluid will accumulate on the surface of the paper without penetration through the paper, thus producing inefficient extraction.

DETERMINATION OF CHOLESTEROL

The method for the determination of cholesterol in blood is the same as described in the original paper.[1] Twenty-five-hundredths c.c. of well-mixed oxalated blood is placed on a filter paper disc. This is best done by placing the paper disc over the cavity of a porcelain plate, such as is used for titration with outside indicators, and distributing the blood from a small pipette over the paper disc by moving the tip of the pipette over the surface of the paper. In this manner the blood is spread over a large area of the disc. The placement of the filter paper over the cavity of the porcelain plate prevents the loss of blood from the under surface of the paper disc were it to penetrate the paper.

The disc containing the blood is now allowed to dry at room temperature for about ten minutes. It may be dried for a short while in the incubator at 37° C., but no higher temperatures should be used as the heat will so affect the paper as to give lower values for cholesterol.

An extraction tube is then filled with about 5 c.c. of pure dry chloroform and the disc containing the blood is picked up with a pair of forceps and placed in the tube containing the chloroform. The same forceps may be used to push the disc down into its place and straighten it out so that it rests over the constricted portion of the tube. One must observe that the disc should lie well beneath the opening of the side arm so as not to obstruct it, as this would prevent the free passage of the chloroform. The tube is then attached firmly to the stopper of the condensing tube and the water-bath is raised to such a height that the extraction tube is immersed in the water to the level of the chloroform. Extraction is continued for half an hour from the time the chloroform begins to boil. When the extract is cool, chloroform is added exactly to the 5 c.c. mark on the tube.

In a similar tube are placed 5 c.c. of cholesterol standard (5 c.c. of chloroform containing 0.4 mg. cholesterol).

To each tube is then added 2 c.c. of dry acetic anhydride. The tubes are placed in a beaker of cold water and 0.1 c.c. of concentrated H_2SO_4 added to each tube. The tubes are then removed from the cold water, tightly stoppered with clean dry cork stoppers and inverted to mix the contents. They are then placed in a dark place for ten minutes to allow the color to develop

*The filter paper discs may be obtained from The Empire Laboratory Supply Company, New York.

and are compared in the colorimeter. The colorimetric matching is done in a somewhat darkened room.

Calculation of Results:

$$\frac{S}{R} \times 160 = \text{mg. cholesterol per 100 c.c. of blood.}$$

S = reading of standard.
R = reading of unknown.

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COLORIMETRIC DETERMINATION OF SMALL AMOUNTS OF ARSENIC IN BIOLOGIC MATERIAL*

By E. H. Maechling, Ph.D., and F. B. Flinn, Ph.D., New York City

EARLY in our investigation as to the effect of arsenic on the skin, we were faced with the fact that the March method was unreliable when used as a quantitative method in determining the minute quantities of the metal present in the samples of blood, spinal fluid, and scales which were received in the laboratory. Observations showed that these results might be as much as 100 per cent out of the way on some of our samples. This led to a study of the literature of the subject. To those interested in such a survey, we recommend the papers by Minot,[1] Kleinmann and Pangritz[2] who have published a comprehensive study of the methods suggested previous to 1927.

Recently Truog and Meyer[3] proposed an improvement on Denigès' colorimetric method for phosphorus and arsenic by means of stannous chloride as a reducing agent. They state that they had been unable to find a systematic study as to the effects of the concentration of sulphuric acid, ammonium molybdate and stannous chloride on the intensity of the color produced. Evidently the work by Kuttner and Cohen[5] on this problem had been overlooked by them.

Denigès[4] pointed out the sensitiveness of the reaction between ammonium molybdate and stannous chloride. Hüttig[6] studied this reaction and concluded that it could be used for the detection and estimation of stannous chloride. Denigès[7] in a later paper discusses the different molybdenum blues in the presence and absence of such reducing reagents as stannous salts, copper, mercury, tin, aluminum, zinc, and hydroquinone. He discusses the differences between the stable and unstable molybdenum blues and came to the conclusion that an ammonium molybdate sulphuric acid solution which had been reduced

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by means of copper filings to a yellow solution containing MoO_3 was the best reagent for the detection and determination of arsenic.[8]

When trying this method out in our laboratory, we discovered that the amount of copper present in the reduced ammonium molybdate sulphuric acid solution affected the results. It is difficult to control the amount of copper which would be brought into solution because it depends on the surface of the copper filings exposed.

After studying the various reducing reagents, we came to the conclusion that hydrazine sulphate was the best reagent for the purpose. It permits the use of definite quantities of the chemicals employed and produces the yellow solution resembling Denigès' reagent containing the lower oxides of molybdenum. The ammonium molybdate sulphuric acid solution reduced by hydrazine sulphate will give a definite though faint blue color when as little as 0.25 microgram of pentavalent arsenic or phosphorus is present. The blue color is permanent for twenty-four hours or longer which is an advantage.

Our improvement of the Denigès method consists in the use of hydrazine sulphate as a reducing reagent which reduces the ammonium molybdate sulphuric acid solution, decomposing during the reduction into gaseous products which are eliminated, before it is added to the solution containing arsenic or phosphorus. An excess of the reducing reagent is avoided which is necessary as it prevents the reduction of the pentavalent arsenic. (See Kubina.[9]) The method depends on the reaction between the pentavalent arsenic and the lower molybdenum oxides. Wu[10] discusses in a very able manner the reactions which take place between molybdenum and pentavalent phosphorus or arsenic.

METHOD

Our method of analysis is briefly as follows:

The dried and weighed sample is destroyed in a Kjeldahl flask by means of concentrated sulphuric and nitric acids in the presence of a drop of concentrated copper sulphate. Care must be taken to avoid charring for as shown by Kesten[11] this will result in a loss of arsenic. With some material it has been found advantageous to use a little peroxide of hydrogen to assist in the combustion. After the wet combustion has been completed, the arsenic is distilled off using the same flask as was used in combustion for the distillation flask. The procedure is that described by Bang.[12] The distilled arsenic chloride is caught in a receiving cylinder containing an oxidizing mixture made up of 15 c.c. of concentrated nitric acid and 5 c.c. of bromine water to convert the arsenic to the pentavalent form. We have found it advantageous to use the Allihn form of condenser and place between the condenser and the cylinder a connecting safety bulb into which some of the oxidizing solution has been drawn. The distillate is placed in a porcelain casserole and evaporated to dryness on a water-bath.

Care is taken not to carry the distillation to the point where SO_2 is carried over. If this should occur, the solution must be neutralized with NaOH after evaporation then made slightly acid with sulphuric acid using phenolphthalein as an indicator. It also requires a new standard arsenic solution, prepared in exactly the same way.

The solutions required for the colorimetric determination are prepared as follows:

A. A 1 per cent hydrazine sulphate solution.

B. Ammonium molybdate sulphuric acid solution. This is prepared by mixing 1 volume of 10 per cent ammonium molybdate solution with an equal volume of concentrated sulphuric acid in 7 volumes of water. To obtain a perfectly colorless solution the ammonium molybdate solution must be added to a cold solution of the sulphuric acid in water.

C. To prepare the molybdate solution 10 c.c. of Solution B and 2 c.c. of Solution A are placed in a test tube and immersed in boiling water for exactly one and a half minutes. The solution should now be yellow and while still warm is poured back and forth between two test tubes to assist in the removal of the gas bubbles which are formed during the reduction. The solution free from gas bubbles is cooled and placed in a glass stoppered container for use. This solution keeps for a week. Before being used it should be checked by adding 0.6 c.c. of the solution to 9 c.c. of water and heating on a hot water-bath for five minutes. No color should develop. On addition of 5 c.c. of a standard solution of arsenic a light blue color appears.

D. Standard arsenic solution: A standard arsenic solution is prepared from chemically pure pentavalent arsenic in such a way that each c.c. of the solution will contain 0.001 of a milligram of arsenic. This solution should be kept in a glass stoppered bottle in a cool place.

Details of colorimetric determination: Nine c.c. of water and 0.6 c.c. of reduced molybdate reagent are added to the usually colorless dry residue from the distillate in a casserole and the casserole is covered with a watch glass and placed on an actively boiling water-bath simultaneously with another casserole containing 5 c.c. of the standard arsenic solution to which 4 c.c. of water and 0.6 c.c. of the reduced reagent C have been added. The standard and unknown samples are kept in boiling water for five minutes. They are then allowed to stand at room temperature for at least half an hour and then transferred to a 10 c.c. graduated test tube and when cold distilled water is added to bring the solution up to the 10 c.c. mark.

The solutions are compared in a micro colorimeter: The amount of reagent used gives accurate results over a range from 0.25 micrograms up to 20 micrograms. To determine larger quantities of arsenic, one must add more of the reagent and a correspondingly larger amount of water. When larger amounts are expected in specimens of biologic material, then the distillate should be made up to a definite volume and only an aliquot part of it used for the distillation and subsequent colorimetric determination.

It is necessary to point out the importance of repeated blank tests for all the reagents used throughout the complete procedure for it has been our experience that even the so-called arsenic-free chemicals frequently contain small amounts of arsenic. Erroneous results would be obtained without this correction when one is determining the small amounts generally present in such biologic specimens as blood and spinal fluid. (Brahme.[13]) The reagents used in our work gave values for the blank up to 3.84 micrograms for the amounts used for a complete analysis.

SUMMARY

1. Hydrazine sulphate is introduced as the reducing reagent.

2. The reduction of the ammonium molybdate sulphuric acid solution is carried out previous to the addition to the solution containing the arsenic or phosphorus.

3. The reducing agent, hydrazine sulphate, is completely decomposed during the reaction and the gases expelled leaving only the lower oxides of molybdenum which combines with the pentavalent arsenic, producing a blue color which is directly proportional to the amount of arsenic present.

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A SMALL PORTABLE ARTIFICIAL PNEUMOTHORAX APPARATUS*

By Minas Joannides, M.S., M.D., Chicago, Ill.

COMPRESSION therapy in certain cases of pulmonary tuberculosis is now beyond the point of experimental stage. In sanitarium and hospital patients, artificial pneumothorax can be induced very easily regardless of

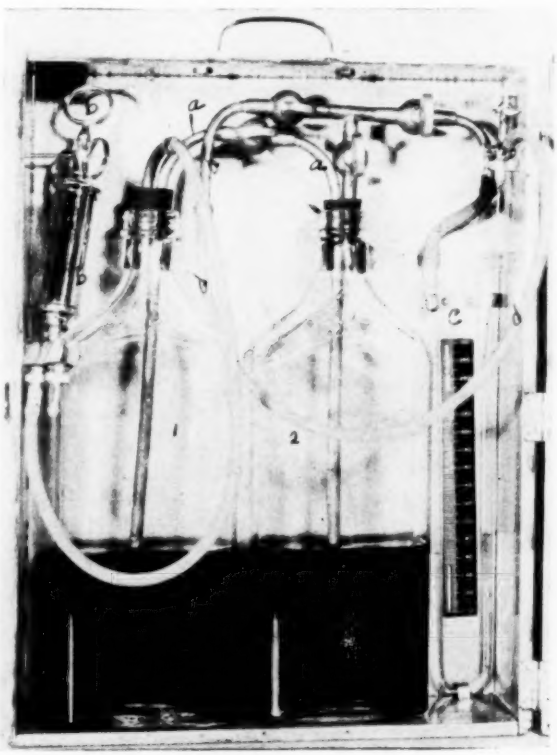

Fig. 1.

what apparatus one may use for this purpose. If, however, one treats ambulatory patients, it becomes necessary to use a handy portable outfit that would not occupy much space in the office or in the automobile. The apparatus described here was designed with this purpose in mind.

It is made up of a very lightweight wooden box which is 14 inches long, 11 inches wide, and 3 inches deep. It contains two 1000 c.c. bottles that are connected to each other by means of a brass tubing "*a*" (see Fig. 1). By

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pumping air or using suction in bottle No. 1, by means of a small Potain aspirator "*b*," we are able to vary the level of the fluids in the two bottles. The air that is injected into the chest comes from bottle No. 2. When the air is pumped into bottle No. 1 by means of the Potain aspirator, the water is pressed into bottle No. 2, and thus the air contained in this bottle is forced into the chest. By using the suction portion of the Potain aspirator we can refill bottle No. 2 with air and also aspirate the chest. A water manometer "*c*" (see Fig. 1) is so arranged that it can record the pressure in the bottles, the pressure in the chest, and also the amount of air injected into the chest. The fluid in the bottles may be plain water or pyrogallic acid, depending on whether one wishes to inject plain atmospheric air or nitrogen. The rate of injection varies with the rate of pumping. Obstruction or dangerous increase in the intrapleural pressure can be detected by the increase in the positive pressure as indicated in the water manometer. With the average compression one needs an average of about 600 c.c. of air. The bottles in our apparatus are large enough to supply even 900 c.c. if ever necessary.

This apparatus can be procured from V. Mueller and Company of Chicago.

DEPARTMENT OF REVIEWS AND ABSTRACTS

ROBERT A. KILDUFFE, M.D., ABSTRACT EDITOR

A SIMPLE OBJECT FINDER: Rosenow, E. C. Science 70: 1809, 1930.

The method consists simply of the use of a blank slide, on one side of which is pasted a fairly thin sheet of gummed paper trimmed accurately along the edges of the slide. On either end of this a circle is drawn with a five-cent piece (2.2 cm. in diameter). When a microorganism or another object of particular importance which is to be photographed or demonstrated later is found, a rough sketch of the high power (H.P.) of oil-immersion field is drawn in the circle at the left end, and a rough sketch of the low-power (L.P.) field in the circle at the right end of the paper-covered slide. In each case, the object of interest is drawn and its position relative to particularly conspicuous material is indicated. Examples of such conspicuous material are masses of pigment, partitions, margins of section, ganglion cells, round cells (R.C.), blood vessels (B.V.), and central canal (C.C.). The same is done if the slide preparation is a smear instead of a section.

The slide in which a field of special interest has been found is then removed from the mechanical stage and the paper-covered slide, on which the high-power and low-power sketches have been drawn, is put in its place; it is necessary to make sure that the mechanical stage is not jarred and that the slide is in the proper position. A dot is then made with pen and ink as nearly as possible in the center of the bright area transmitted from the condenser. The low-power lens is then focused on the dot and its situation in the field noted. The paper-covered slide is then removed and the situation of the dot in relation to the field is indicated with a small circle around the dot. The paper covered indicator slide is then numbered to correspond to the slide containing the prized field and both are filed away together for future reference. If more than one interesting field is found in the same slide, any number of additional indicator slides may be used.

To find the particular field or fields later, the indicator slide is put in place, and the dot brought to the relative position in the low-power field as indicated by the circle surrounding it. The slide containing the object is then put in its place, the low-power field is oriented, and the object of interest brought to the center of the field. The high-power or oil-immersion lens is then turned into position and the prized object is almost always within the field, and if not it can readily be located.

GONORRHEA: Recent Advances in the Treatment of, White, C., and Winter, H. G. J. Roy. Army M. Corps. 53: 250, 1929.

Exhaustive and extensive considerations of the biochemistry and metabolism of the gonococcus showed that when the gonococcus, or any other organism, was grown on a medium rich in nucleo-protein, it tended to produce polar bodies which were loosely attached to the organism; these bodies turn black with Neisser's stain and are easily distinguishable. They were found to consist of equal parts of alpha-nucleo-protein and beta-nucleo-histone, the latter portion being soluble in 2 per cent salt solution. The culture media used were found to be of exceptional value, and are described below. The routine preparation of the vaccine is as follows: Large 6 x ¾ inch tubes of the nucleo-protein medium are inoculated with gonococcal culture and are incubated for twenty-four hours. In order to insure uniform growth, the same sized tubes, sloped to the same angle, and a standard inoculating loop are used. After twenty-four hours each tube is washed with 1.5 c.c. of 2 per cent saline carbolized with 0.5 per cent phenol, a separate pipette is used for the addition of the saline and for removal of the emulsion from the tubes, thus insuring that the stock carbolized saline is not con-

taminated with gonococcal bodies which might autolyse on standing and release their endotoxin. The emulsion is put in vaccine bottles in quantities of 25 c.c. Periodical counts show the average content to be 7,000 x 10^6 per c.c. These bottles are put, with the least possible delay, in a high speed centrifuge giving 9,000 revolutions per minute and are "swung" for about four minutes. After centrifugation it will be noted that the contents have separated into three layers, a lower grayish layer consisting of the bodies of the gonococci, a middle cream-colored layer of the alpha-nucleo-protein element of the polar bodies and a clear supernatant fluid which is a saturated solution of the beta-nucleo-histone in a 2 per cent carbolized saline. This clear fluid is pipetted off and put in vaccine bottles ready for use.

The vaccines are given intradermally.

The greatest care must be exercised to carry out the whole process of production under the strictest aseptic precautions as sterilization by heat will only result in the destruction of several important properties.

APPARATUS

1. Test tubes: The most convenient size is 6 inches by ¾ inch. These are boiled in the following solution:

Potassium chromate	50 gm.
Sulphuric acid, commercial	60 c.c.
Tap water	500 c.c.

for two hours and then brushed and rinsed in hot water three or four times and left to soak in water for two hours; next dried in a hot air oven, plugged and sterilized at 160° C. for one hour.

2. Filler. This is sterilized at 120° C. for one hour.

3. Flasks. Fill with strong solution of potassium permanganate and leave for twenty-four hours, wash with hot water followed by commercial hydrochloride acid. When all stains are removed the flasks are rinsed in hot water to render them acid free, dried in the hot air oven, plugged and sterilized at 160° C. for one hour.

MEDIA

There are two types of media employed: (1) Isolation medium. (2) Nucleic acid medium.

The isolation medium is essentially a nutrient serum agar, while the nucleic acid medium contains in addition a thymus nucleic acid base. They both contain an alkaline autolysate of ox heart.

A. Alkaline Autolysate of Ox Heart.—Obtain the hearts fresh, free them from all trace of fat and fibrous tissue, cut the meat into small cubes and mince in a sterile mincer. Weigh the minced heart and place in a sterile pan. To every gram of meat add 1 c.c. of distilled water and, in the case of fresh hearts, 40 c.c. of N/1 NaOH to every liter of water added. In the case of frozen hearts 42.5 c.c. of N/1 NaOH.

Leave the whole in the ice chest overnight to macerate. Next morning strain through gauze in 500 c.c. bulks into liter flasks. Steam for quarter of an hour to allow coagulation to take place.

Strain again and steam for fifteen minutes on the first day, five minutes on each of three successive days.

B. Agar Base—

Witte's peptone	6 per cent
Agar fiber	6 " "
Sodii chloride	1 " "
Sodii phosphate	1 " "
Aqua dist.	q.s.

This base is made up in 200 c.c. bulks in 500 c.c. flasks and is placed in a steamer until the agar is melted, then to each add 0.3 per cent of glucose and steam for a further fifteen minutes.

The base is made up the day before the batch is to be tubed off.

C. Herring-Roe Extract.—Take ripe sperm from herring, grind it into a paste with distilled water, adding to every gram of roe 1 c.c. of water. Strain through gauze, steam for half an hour on three successive days.

In an emergency, tinned roes may be used (Noel and Sons are the best), but the resulting yield of bodies is not good.

D. Nucleic Acid Base—

Dessicated thymus	4 gm.
Nucleic acid	1 gm.
Aqua dist.	100 c.c.

Mix in 250 c.c. flask and steam for fifteen minutes. Adjust the P_H to 7.2 as follows: Add 15 c.c. of 0.02 per cent phenol red solution and 100 c.c. of herring-roe extract then add N/1 NaOH until the yellow color turns to brownish yellow, steam for a further ten minutes. Note: If it is heated too much there is apt to be an alteration in the P_H and consequent loss of efficiency of the medium.

I. ORDINARY ISOLATION MEDIUM

Steam the agar base (made as in *B* above, but without glucose) until the agar has melted. Steam the heart extract (*A* above) for five minutes. Mix 200 c.c. of agar base with 200 c.c. of heart extract. Tube off 10 c.c. to a tube, add 0.5 c.c. of human serum to each tube and slope.

II. STANDARD OR NUCLEIC ACID MEDIUM

Take ager base 200 c.c. and steam until melted. Heart extract 50 c.c. and steam for five minutes, nucleic acid base 200 c.c. and steam for fifteen minutes.

Mix all together, tube off 15 c.c. to a tube and to each tube add 0.5 c.c. of human serum. Slope.

The following brands of ingredients have been found to give the best results:

Witte's peptone must be Bostock German brand. Sodium phosphate, British Drug Houses, pure. Nucleic acid, Martindale's. Thymus, dessicated, Willows, Francis, Butler, and Thompson.

BLOOD STAIN: Rees, C. W. Science **71**: 1831, 1930.

Rees reports the following method of fixation as enabling the use of Giemsa's stain and iron-hematoxylin for the demonstration of intracellular parasites.

After drying the smears are immersed in Schaudinn's alcoholic sublimate solution without acetic acid.

Good results may also be obtained by first placing the smears in a Coplin jar containing about 3 c.c. of 40 per cent formaldehyde and then treating with the alcoholic sublimate solution. This is especially useful when the work is done where the humidity is high.

SYPHILIS: The Controlled Flocculation Test for. Michailoff, A. Am. J. Hyg. **11**: 202, 1930.

The antigen is prepared from fresh veal heart freed from fat and blood vessels and passed through a meat grinder. The muscle is put in a large flask and 95 per cent alcohol added in the proportion of 125 c.c. of alcohol to 100 gm. of muscle. The flask is kept at room temperature for twenty hours, then the alcohol is filtered off and the muscle spread between several sheets of filter paper and gently pressed to dry it. It is necessary to change the filter paper several times until no more moisture is taken up by it. After about an hour the muscle is sufficiently dry to be put in a large dry flask and acetone added in the proportion of 200 c.c. of acetone to 100 gm. of muscle. A guinea pig is then killed, its brain freed from membranes and vessels, cut into small pieces and 2 gm. of brain are added for each 100 gm. of muscle in the acetone. This is placed in the incubator at 37° C. for forty-eight hours and frequently shaken. Filter off the acetone, dry between filter paper as before. Then again pass the muscle-brain mixture through a fresh solution of acetone in the same

proportion as before, being sure to use a clean dry flask for the fresh acetone. The mixture should remain in the incubator for forty-eight hours and shaken frequently. Again the acetone is filtered off and the muscle-brain tissue dried between several sheets of filter paper. The above-described steps are merely to remove the anticomplementophylic substances and are followed by the true extraction. The muscle-brain tissue, dried and hardened by the above-mentioned operations is now placed into absolute alcohol in the proportion of 200 c.c. of absolute alcohol to 102 gm. of tissue in a large, dry, well-stoppered flask. The flask is kept in the incubator at 37° C. for six days, and shaken very often. On the seventh day the supernatant liquid should have a yellowish color. Filter the liquid through two sheets of filter paper and stopper the flask well. The next day filter again. This filtered liquid is the antigen which shows no tendency to self-precipitation and in well-stoppered dark flasks can be used for more than a year. This antigen is not anticomplementary nor hemolytic in a dilution of 1 to 5 and if properly prepared can be used in a dilution of 1 to 20 with 8.5 per cent saline, or 1 to 30 for the regular Wassermann test without antigen titration. No further manipulation is necessary for its use as the antigen in the Bordet-Ruelens reaction.

Technic of the Test—

The antigen is diluted immediately before using in the proportion of 1 c.c. of antigen to 20 c.c. of 8.5 per cent saline. It is very important to add the saline to the antigen in drops while shaking constantly.

The hemolysin: A small quantity of complement should be used. The author had best results with 4 hemolytic units. Titration was done with 0.5 c.c. of a 5 per cent solution of washed sheep cells and 0.5 c.c. of 1/100, 1/200, 1/400, etc., dilutions of hemolysin in the presence of 0.2 c.c. of 1 to 10 diluted complement collected twelve hours previously. If 0.5 c.c. of a 1/3200 dilution of hemolysin produced complete lysis in thirty minutes, we used equal parts of 1/800 dilution of hemolysin and 5 per cent sheep cells as our hemolytic system.

Complement titration: This should be done daily with 1 c.c. of the fresh hemolytic system and 0.05, 0.1, 0.15, 0.2, 0.25, and 0.3 c.c. of 1 to 10 complement dilution in the presence of 0.5 c.c. of saline. Ordinarily 0.1 c.c. or 0.125 c.c. is the amount necessary to produce complete hemolysis in thirty minutes. This amount of the complement dilution is used in the reaction but of the hemolytic system they use only 0.5 c.c. (the equivalent of two hemolytic units of complement).

The test: For the flocculation test or the controlled flocculation test take two tubes 1 cm. in diameter and 12 cm. long. In the first tube put 0.2 c.c. of the diluted antigen. To both tubes add 0.2 c.c. of the serum which is to be examined and which has previously been inactivated by keeping it at a temperature of 56° C. for thirty minutes. Now add two units of complement to both tubes and sufficient saline to bring the volume to 1.0 c.c. The addition of complement is facultative and therefore the test can be done as a flocculation test where complement is not available.

Shake both tubes gently for exactly four minutes. For this purpose a shaking apparatus is desirable in which the racks must be placed in an inclined position. This tends to lessen the foaming and bubbling of the solution. Flocculation often occurs, with or without complement. Ability in reading depends upon experience and is facilitated by the use of an agglutinoscope. The reaction becomes more evident after awhile and after twenty-four hours at room temperature a large floccule, easily observed, is to be seen in all sera positive to the Wassermann test.

The addition of complement and the control of complement absorption gives a much more definite result, one that is easier to read and more time saving. The latter method requires only about an hour for its completion, and is done as follows: In addition to the directions given above for the preparation of the simple flocculation test with complement one additional step is necessary; i.e., to each of the two tubes is added 0.5 c.c. of the hemolytic system previously referred to. Shake the two tubes well and let stand in a water-bath at 37° C. Hemolysis ordinarily occurs after fifteen minutes. Anticomplementary sera hemolyze slowly or not at all. The result is read five minutes after the control tube shows complete hemolysis as in the ordinary Wassermann test. The end-reaction is stable for at least an hour at room temperature. If the reaction is not evident at the end of thirty

minutes or if the control tube does not show marked hemolysis in fifteen minutes the serum should be considered more or less anticomplementary and the results false. The reaction should then be repeated using 4 to 8 complement units. To avoid anticomplementary reactions in sera, separate the serum from the clot, shaking the serum for five minutes, then centrifuge, and finally inactivate.

Cerebrospinal fluid is examined by the same technic using 0.5 c.c. of noninactivated fluid, but omitting saline.

AMEBA: Study of Stools Cultured for E. Histolytica, Tripoli, C. J. Am. J. Med. Sc. **178**: 682, 1929.

The following method was used:

Ingredients of Media: (*a*) Serum-Ringer Solution: Beef or human blood is obtained. After clotting it is placed for twenty-four hours in a refrigerator. The clear serum is pipetted off and mixed with Ringer's solution in the proportion of one part of serum to eight parts of Ringer's solution. Sterilization is accomplished by passing the fluid through the large type Seitz-Wertz filter, which permits the fluid to pass only through the filter pad. Filtration is accomplished by use of full vacuum. The filtered, sterile, diluted serum is immediately pipetted off with a 50 c.c. sterile pipette and placed in sterile flasks 50 c.c. to each flask. These flasks are kept at 8° to 10° C. until ready for use. Inactivation of the mixture (56° C. for thirty minutes) is not necessary.

(*b*) Starch. Rice starch possesses the necessary uniformly small grains which are readily ingested by the amebas, and further is not easily hydrolyzed when suspended in fluid.

The following method of sterilization was developed which apparently fulfills the requirements and has given entire satisfaction:

The starch is weighed out in 0.2 gm. quantities, placed in small soft filter paper tubes which are made by rolling a $3\frac{1}{2}$ by 5 cm. piece of thin filter paper about a pencil and in which the starch is kept in place by lightly crimping the ends of the paper tube. The filled tubes are placed in test tubes which are then plugged with cotton and covered with several layers of wrapping paper which is well tied down over the end of the tube, and the whole assembly autoclaved at 15 pounds pressure for fifteen minutes. After autoclaving the paper covering is removed and the tubes are placed in a hot air oven or incubator to evaporate the little moisture that may have condensed upon the walls of the tube.

(*c*) Acriflavin Solution: A 1 per cent solution of acriflavin is prepared and sterilized in the Arnold sterilizer. Acriflavin solution keeps fairly well when protected from light and at a low temperature.

(*d*) Egg Base: Four eggs are emulsified with 50 c.c. of normal saline and poured into $1\frac{1}{2}$ by 15 cm. tubes to a depth of approximately 2 cm. The tubes are placed upright in the water-bath and heated to 80° C. for sufficient time to coagulate to a firm consistency, after which they are autoclaved at 15 pounds pressure for fifteen minutes in the upright position. Amebas grow best upon a flat surface.

Assembly of Media: (*a*) the starch is added (0.2 gm.) to the serum-Ringer solution (50 c.c.) by placing the small paper tubes into the flask. Slight agitation opens up the paper and liberates the starch.

(*b*) Acriflavin is added by transferring 0.1 c.c. of the 1 per cent solution to 50 c.c. serum-Ringer starch, which gives an ultimate dilution of 1 to 50,000. Some samples of acriflavin require a greater concentration (1 to 25,000) to retard the growth of unfavorable flora.

(*c*) Four to 5 c.c. of sterile serum-Ringer's solution plus the starch and acriflavin are then poured into the tubes containing the coagulated egg. The tubed culture media should be stored in the refrigerator until used. At the time of inoculation the media should be warmed to 37° C. An equal number of tubes should be prepared, omitting the acriflavin.

The P_H of the prepared media varies from 7.2 to 7.8 and needs no adjustment, this being the optimum range of hydrogen-ion concentration determined for E. histolytica.

Drbohlav advocates the addition of 5 gm. per liter of potassium acid phosphate to the media which is adjusted to a P_H of 7.4. The authors have found this to be unnecessary as

the medium is capable of adjusting itself to the original P_H even after forty-eight hours of bacterial growth which tend to form organic acids.

Method of Inoculating, Examination, and Transplanting Cultures: A particle of fecal material about 1 or 2 mm. in diameter is picked up on a wooden applicator and is transferred to a tube of plain, and a tube of acriflavin charged media which have been warmed to about body temperature.

With soft or dysenteric stools, which are more apt to contain vegetative amebas, the material used for inoculation should be obtained fresh from the patient. Proctoscopic removals and rectal washings should, similarly, be inoculated immediately.

With formed or hard stools, which are more apt to contain cysts, the cold stool will suffice even though it be several days old. The same results were obtained with cysts fresh from the patients or with those kept in the refrigerator several days.

The tubes are incubated in an upright position at 37° C.

Maximum growth is usually seen on the third or fourth day of incubation. On the fifth or sixth day the amebas usually die unless they are transplanted into fresh media. Under cultural conditions very few amebas encyst, but with frequent transplantation they can be propagated in the vegetative form for an unlimited period.

Examination of cultures is made by skimming the débris of starch and bacteria from the surface of the coagulated egg with a capillary pipette having a large lumen (1 mm.) which has been scratched and broken to present a square tip. Use a Wright's rubber bulb to produce suction. A fraction of a drop of material is removed, placed on a slide, cover glass added, and examination made with the 16 mm. objective.

To transplant the culture a drop of the material is transferred to fresh media that has been previously warmed.

BACTERIA: Negative Staining for, Dorner, W. C. Stain Tech. **5**: 25, 1930.

Boil 10 gm. of nigrosin in 100 c.c. water about thirty minutes. Filter several times through the same filter paper, adding 10 drops of formalin before the last filtration as a preservative. Place a small loopful of this solution on a clean slide and add the bacteria with a needle or loop. After mixing, spread the mixture a little irregularly on the slide and dry either at room temperature or slowly over a low flame, the preparation being examined in oil immersion without the use of a cover slip.

MENINGOCOCCI: Preservation of Cultures, Bourguignon, G. C. Ann. Soc. Belge de méd. trop. **9**: 59, 1929.

The death of meningococcus in culture, necessitating daily subculture in order to maintain it alive, is attributed to rapid increase of alkalinity and to the presence of too much oxygen. An ascitic fluid medium, containing 0.2 per cent glucose, and covered with a layer of vaseline has been used for the preservation of gonococci. The glucose by its fermentation retards the increase in alkalinity. This medium has been found by the author equally useful for the preservation of both meningococci and gonococci, which have been kept alive for thirty-seven days without subculture.

INFLUENZA: Study of Hemophilus Influenzae, Evans, M. J. Am. J. Med. Sc. **179**: 177, 1930.

The virulence of cultures remains unimpaired for as long as two years if kept at room temperature (18° to 20° C.) in infusion broth P_H 7.6 to 7.8 to which is added approximately 7 per cent by volume of sterile defibrinated blood; the whole then heated until it becomes a rich brown color.

EOSINOPHILIA: In Liver Diet, Mullengracht, E., and Holm, S. Am. J. Med. Sc. **179**: 199, 1930.

Eosinophilia in liver treatment of pernicious anemia has appeared in a marked and persistent form when the treatment is carried out with raw liver (calf) in large doses. As

a rule, the eosinophilia has appeared rather suddenly after about four weeks of treatment, and it has reached to high degrees, 20, 40, and even 74 per cent. It seems to persist as long as the administration of raw liver is kept up. On treatment with fried liver (calf) or liver extract, the phenomenon has usually been absent; and when present in single instances, it was in a faint and transitory form.

Control individuals suffering from various other diseases have responded to the treatment in the same manner as have patients with pernicious anemia, as they constantly showed eosinophilia on ingestion of raw liver but not after intake of fried liver or liver extract.

This eosinophilia is to be considered a by-product in the treatment of pernicious anemia with raw liver that has nothing to do with the curative effect of the treatment.

As far as directly observable, the eosinophilia represents a harmless phenomenon.

MORGAN'S BACILLUS: Infections Probably Due to, D'Aunoy, R. Am. J. Med. Sc. 178: 834, 1929.

The isolation of Morgan's bacillus in cases of pyrexia, pyelitis, and colitis is reported. One strain isolated proved pathogenic for small laboratory animals. Agglutinins were demonstrated for homologous organisms in the blood of all three patients. Observations upon some biologic characteristics of the organism are presented together with a plea in support of Thjotta's position for noninclusion of the organism in the genus Salmonella.

DEXTROSE: Preparation of Dextrose for Intraperitoneal Injection, Shohl, A. T., and Beal, F. Am. J. Dis. Child. 38: 943, 1929.

Five grams of chemically pure, finely ground dextrose are weighed and placed in a test tube approximately 4 x ¾ inches (100 mm. by 15 mm.) and the test tube is loosely stoppered with a cotton plug. This is inverted and placed in a wide-mouthed bottle containing from 25 to 50 gm. of granular anhydrous calcium chloride. The bottle is fitted with a cork and autoclaved at 15 pounds (6.8 Kg.) for twenty minutes. This preparation will keep for at least several months.

For use, we make the solution with freshly distilled water. It is taken directly from the still and boiled five minutes (but not redistilled). One hundred c.c. is poured on the sterile dextrose which has been placed in the graduated flask to be used for the injection, and a 5 per cent solution is thus made. It is given intraperitoneally with the usual precaution, at body temperature.

BLOOD CHEMISTRY: The Accurate Determination of Chlorine and Iron in Blood and Other Liquids, Smirk, F. H. Biochem. J. 22: 201, 1928.

The pipette used is drawn from thick capillary tubing, and a length of 2 or 4 cm. is almost sealed off from the main body by means of a constriction; that length should hold from about 0.01 to 0.02 c.c., the actual volume from the top to the constriction being determined by weighing mercury from it. For chlorine, the pipette is filled with serum, whole blood, corpuscles, urine or sweat, and the fluid is delivered to the bottom of a test tube; the pipette is washed three times with distilled water, the washing water being taken from a drop at the end of the burette and the washings added above the liquid in the test tube. The pipette is cleaned, filled with silver nitrate solution of the strength appropriate to the solution under analysis, and washed with nitric acid. A little powdered ammonium persulphate is added, and the solution is heated until it is clear. It is then cooled, and an equal volume of acetone and a drop of saturated iron-alum solution are added. The solution is titrated with alcoholic ammonium thiocyanate. The first red flush is taken as the end-point; 0.03 c.c. thiocyanate, added after this, should make the solution distinctly red. The acetone diminishes the dissociation of the ferric thiocyanate and makes the end-point sharper. For iron in the blood or corpuscles, the same pipe is used, and concentrated nitric acid and ammonium persulphate are added to the liquid, which is then heated, cooled, and diluted with 50 per cent acetone; ammonium thiocyanate solution is added, and the color is compared colorimetrically with a standard.

REVIEWS

Books for Review should be sent to Dr. Warren T. Vaughan, Medical Arts Building, Richmond, Va.

*The Common Head Cold**

THIS is an exposition of the causes, prevention and treatment of acute coryza, for popular consumption. It is written from the point of view of the rhinologist.

The author justifies the length of his monograph by the observation that more than ninety million workdays are lost each year on account of this malady, with a total economic cost of four hundred and fifty million dollars. Considerable space is devoted to discussions of anatomy and physiology. The major portion of the volume deals with preventive measures which in the last analysis develops into a discussion of personal hygiene. The author's therapeutic measures for home administration are rational and in keeping with our present-day understanding of the problem. He appears to have no pet hobbies.

Botany†

AN INTRODUCTORY textbook on general college botany. Since with many undergraduate students the only formal work in the biologic sciences is the study of botany, these authors have included a general presentation of fundamental biologic principles. In other words this volume is a textbook of biology, in which representatives from the vegetable kingdom are used for illustrative purposes. The illustrations are most abundant and are clearer and more instructive than in many other books dealing with this subject.

Tuberculosis‡

A MANUAL for the use of the patient or his family, on details of home treatment. We can recommend it for this purpose, especially for the patient or family of only moderate intelligence. The more highly educated or intelligent patient will probably want a book of greater detail.

We take exception to the author's statement on page 4, that tuberculosis is not an infectious disease. He explains this statement but the explanation does not satisfy. In the United States there might be some misunderstanding of the statement on page 41, that the bedroom should be preferably on the first floor. Later we find that the higher the bedroom is removed from the ground the better for the patient. This apparent contradiction in the United States, would disappear in England and on the Continent, where our second floor is termed the first floor.

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*The Common Head Cold and its Complications. By Walter A. Wells, A.M., M.D., F.A.C.S., Prof. of Otolaryngology, Georgetown University, Washington, D. C. With an Introduction by Hugh S. Cummings, M.D., Surgeon General, United States Public Health Service. Cloth, pages 225. The Macmillan Company, New York, 1929.

†Botany. A textbook for College and University Students. By William J. Robbins, Professor of Botany, University of Missouri and Harold W. Rickett, Associate Professor of Botany, University of Missouri. Cloth, pages 535. D. Van Nostrand Company, Inc. New York, 1929.

‡Oxford Medical Publications. Tuberculosis. Its Prevention and Home Treatment. A Guide for the Use of Patients. By H. Hyslop Thomson, M.D., D.P.H., County Medical Officer of Health, School Medical Officer and County Tuberculosis Officer for Hertfordshire; formerly Medical Superintendent, Liverpool Sanatorium and Medical Superintendent Consumption Sanatorium of Scotland, Bridge of Weir. Cloth, pages 99. Humphrey Milford, Oxford University Press, American Branch, New York. Third Edition.

NOTE: In so far as practicable the book review section will present to the reader (a) interesting knowledge on the subject under discussion, culled from the volume reviewed, and (b) description of the contents so that the reader may judge as to his personal need for the volume.

We trust that the scientific information printed in these pages will make the reading thereof desirable per se and will thereby justify the space allotted thereto.

*The Clinical Aspects of Venous Pressure**

IN THIS volume Dr. Eyster reviews the literature and presents his own experimental work on venous pressure. He brings out the increasing importance of clinical studies of the venous pressure, emphasizing that such studies clinically give information concerning beginning congestive failure earlier than any other method. Studies of the venous pressure in cardiacs will therefore probably develop into an important clinical method of control. The author has developed a satisfactory instrument for the clinical determination of venous pressure which will probably come into general use particularly among cardiologists.

*Some Principles of Minor Surgery***

THE author, feeling that while major surgical principles have received wide attention in textbooks, minor surgery is too often glossed over, has devoted a small volume to the latter alone. The work includes the modern surgical treatment of such conditions as clean and infected wounds, abscess, infections of the hand, sprains, fractures, urinary retention, skin tumors, hemorrhoids, ingrowing toe nails, varicose veins, hydrocele, and the like.

Pharmaceutical Latin†

A VEST pocket Latin grammar written primarily for the student who has had no previous acquaintance with Latin, in which the vocabulary is almost entirely confined to Latin words used in prescription writing. Chapters are devoted to Latin phrases and abbreviations used in prescriptions, to the writing of prescriptions, and there is quite a complete Latin-English vocabulary which again includes the Latin words customarily employed in prescription writing.

Pharmacology and Therapeutics‡

A POCKET sized handbook on the pharmacology of the common drugs. The field of usefulness of this volume is limited almost entirely to the beginning student of pharmacology. This is indeed the purpose for which its author indicates that it was intended.

Bacteriology, General, Pathologic, Intestinal§

THE third edition of Kendall's work has been widely revised and brought strictly up-to-date. One may look upon this volume as one on applied bacteriology, rather than pure bacteriology. While it is entirely suited to use as a textbook in the bacteriologic laboratory, the discussions are developed around the diseases caused by bacteria rather than around individual bacteria as a cause for disease. The author devotes considerable space to the chemistry of bacteria, bacterial metabolism, pathogenicity, resistance, and immunity. There is a very interesting chapter on gastrointestinal bacteriology.

This book should be highly recommended both as a textbook and as a reference manual.

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*The Clinical Aspects of Venous Pressure. By J. A. E. Eyster, B.Sc., M.D., Professor of Physiology, University of Wisconsin, Associate Physician, Wisconsin General Hospital, Madison, Wisconsin. Cloth, pages 135. The Macmillan Company, New York, 1929.

**Oxford Medical Publications. Some Principles of Minor Surgery. By Zachary Cope, M.S., M.D.(Lond.), F.R.C.S.(Eng.), Surgeon to St. Mary's Hospital, Paddington, and to the Bolinbroke Hospital. Cloth, pages 159. Humphrey Milford, Oxford University Press, American Branch, New York, 1929.

†Aids to Pharmaceutical Latin. By G. E. Trease, Ph.C., Lecturer in Pharmacognosy, University College, Nottingham, Joint Author of "The Chemistry of Crude Drugs." Cloth, pages 168. William Wood and Company, New York.

‡An Introduction to Pharmacology and Therapeutics. By J. A. Gunn, M.D., D.Sc. (Edin.); M.A.(Oxon.), Professor of Pharmacology in the University of Oxford and Fellow Balliol College; sometime Examiner in the Universities of Belfast, Bristol, Cambridge, Cardiff, Edinburgh, Leeds, Liverpool, London, Oxford, Sheffield, and to the Royal College of Physicians. Cloth, pages 220. Humphrey Milford, Oxford University Press, American Branch, New York, 1929.

§Bacteriology, General, Pathological and Intestinal. By Arthur Isaac Kendall, B.S., Ph.D., Dr.P.H., Professor of Research Bacteriology in the Northwestern University Medical School, Chicago, Illinois. Third edition thoroughly revised. Illustrated with 103 Engravings and 8 plates. Cloth, pages 733. Lea & Febiger, Philadelphia, 1928.

*Organic Chemistry**

AS A textbook of organic chemistry this volume covers the usual field and very much in the customary sequence. Its chief interest to physicians lies in its containing details of the chemical constitution and formulas of most of the synthetic organic chemicals that have come into use of late years in the treatment of disease. With new drugs being introduced each year and the older ones being served to us under a variety of trade names, it would seem practically impossible for such a volume to be up to the minute. However practically all of those which have established a definite place for themselves in therapeutics up to the time of printing will be found. It should be of some comfort to the physician to know that somewhere there is a volume to which he can refer to find out precisely what he is injecting into his patient.

Materia Medica†

THIS is essentially a laboratory manual on the analysis of drugs and poisons for undergraduate use and instruction.

Sterilization for Human Betterment‡

THIS is a most interesting report of the results of six thousand sterilization operations in California between 1909 and 1929. All who are interested in this phase of eugenics should have this volume available.

As the authors point out in their introduction, the human race has reached the present stage of its development by the survival of the fittest. The weak and defective have perished. Modern civilization, human sympathy, and charity tend to interfere with nature's plan. The weak and defective are now nursed to maturity and produce their kind. Under nature's law we bred principally from the top. Today we breed both from the top and the bottom, but more rapidly from the bottom.

The authors estimate that 4 per cent of the people of the United States will at some time need the care of an institution for mental diseases. One-half per cent of our population has a mentality which never passes that of seven years. One per cent has a mentality of from seven to nine years. The moron class, by far the more dangerous, represents about 5 per cent of the population.

One in twelve of the insane admitted to state hospitals in California since the sterilization law was passed have been sterilized. More recently one in five or six of the new admissions is sterilized. Of 6,255 sterilizations there have been three known failures in the male and four in the female. There were four deaths following operation.

The authors find that sterilization as practiced has no deleterious effects upon the sexual life, indeed actual improvement is reported by one man in seven and by one woman out of three. They find no increase in delinquency. This is of course due in great measure to the careful supervision of paroled patients. Sterilization has not been found to increase the tendency to rape. The authors conclude after careful investigation that sterilization of the type of individual that has been subjected to this treatment incurs no risk of loss of good stock, or of prevention of the birth of genus.

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*Organic Chemistry. For Students of Pharmacy and Medicine. By A. H. Clark, Ph.G., B.Sc., M.D. Professor of Chemistry, University of Illinois School of Pharmacy; Member of the General Committee of Revision of the Pharmacopoeia of the United States; Past President of the American Conference of Pharmaceutical Faculties (American Association of Colleges of Pharmacy). Cloth, pages 446. D. Van Nostrand Company, Inc., New York, 1929.

†Practical Materia Medica. An Introductory Text to the Study of Pharmacology and Therapeutics Designed for Students of Medicine. By Clayton S. Smith Ph.D., M.D., Professor of Physiological Chemistry and Pharmacology in the College of Medicine of the Ohio State University, Columbus, Ohio, and Helen L. Wikoff, Ph.D., Instructor in Physiological Chemistry and Pharmacology in the College of Medicine of the Ohio State University, Columbus, Ohio. Cloth, pages 300. Lea & Febiger, Philadelphia, 1929.

‡Publication of the Human Betterment Foundation. Sterilization for Human Betterment. A Summary of Results of 6,000 operations in California, 1909-1929. By E. S. Gosney, B.S., LL.B., and Paul Popenoe, D.Sc. Cloth, pages 202. The Macmillan Company, New York, 1929.

*The Principles of Electrotherapy**

NOWADAYS when one picks up a book on electrotherapy one anticipates finding an elated discussion of the cure of all maladies and, on the flyleaf an advertisement for certain electrical apparatus.

The present volume is not that kind. It is the only work that we have come across recently that puts down intelligently, and for what it is worth, the modern clinical application of electrotherapeutic measures. We can recommend it to those who are interested in this phase of therapy.

Colloid Chemistry†

THE most interesting presentation of the facts of colloid chemistry known today, readily understandable to anyone who has studied general chemistry. In the first seventy-five pages the nature and general properties of colloids is discussed. The next one hundred and sixty pages describes the practical applications of colloid-chemical principles in the various fields of science and the arts, covering such diversified subjects as astronomy, perfumes, geology, dyeing, silk, insecticides, sewage disposal, photography, paper, rubber, ice cream, confectionery, glasses, boiler scale, plaster, pharmacology and therapeutics, antiseptics, biology and medicine.

The last portion of the work consists of suggestions for experimental laboratory demonstrations in colloid chemistry, using substances which are usually available in every household.

The value of this book lies not only in its relatively nontechnical presentation of the subject, but also its availability as a reference manual and its description of practical applications of colloid chemistry.

Practice of Medicine‡

A TEXTBOOK of the practice of medicine by various authors, edited by Frederick W. Price, has since its first publication in 1922 gone through three editions with eight printings. The chief change from the first edition reviewed some time ago in these columns consists in the inclusion of new subjects such as tetra-ethyl lead poisoning, carbon monoxide poisoning, basal metabolism, polyglandular syndrome, internal secretion of the sex glands, intestinal carbohydrate dyspepsia, biliary colic without gallstones, sickle cell anemia, cardiac infarction, the veronal habit, malaria treatment of syphilis, the use of plasmochin in malaria, the laboratory tests for smallpox, diagnostic test of drunkeness, barium therapy and many others of the newer subjects.

This text appears to have successfully supplanted Osler's system, in England. Printed on India paper, the nearly 1900 pages make a not at all unwieldy volume.

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*Oxford Medical Publications. The Principles of Electrotherapy and Their Practical Application. By W. J. Turrell, M.A., D.M., B.Ch.(Oxon.), D.M.R. & E.(Cantab.) Consulting Physician, Oxford County and City Mental Hospital; Physician in charge of the Physiotherapy Department, Radcliffe Infirmary, Oxford; Major R.A.M.C.T.; Late Medical Officer in charge of the Physiotherapy Department, Third Southern General Hospital, Oxford; Ex-President, Electro-Therapeutic Section, Royal Society of Medicine; Honorary Fellow of the American Electro-Therapeutic Association; Honorary Fellow, American Academy of Physiotherapy. Second Edition. Cloth, pages 413. Humphrey Milford, Oxford University Press, American Branch, New York, 1929.

†Industrial Chemical Monographs. Colloid Chemistry; Principles and Applications. By Jerome Alexander, M.Sc. Consulting Chemist and Chemical Engineer. Past Chairman, Committee on the Chemistry of Colloids. (National Research Council); Fellow, Amer. Assn., for the adv., of Science, and Amer. Inst. of Chemists; Mem. Amer. Inst. of Chem. Engineers; Amer. Inst. Mining and Metallurgical Engineers, etc. Third Edition. Cloth, pages 270. D. Van Nostrand Company.

‡Oxford Medical Publications. A Textbook of the Practice of Medicine. By various Authors. Edited by Frederick W. Price, M.D., F.R.S. (Edin). Consulting Physician to the Royal Northern Hospital; Physician to the National Hospital for Diseases of the Heart, London; formerly Physician and Honorary Pathologist to the Mount Vernon Hospital for Consumption and Diseases of the Chest, and Examiner in Medicine at the University of St. Andrew's. Third Edition. Pages, 1871. Humphrey Milford, Oxford University Press, American Branch, 1929.

*An Introduction to the Study of Human Anatomy**

THIS is a laboratory guide for dissection, in use at Washington University St. Louis. As we review this volume we are struck with the observation that the student is taught to critically survey and analyze those things which he is inspecting and handling and to do this with an inquisitive point of view.

In other words this volume appears to be an attempt in so far as such is possible in cold type to reproduce the finer type of the human demonstrator in anatomy, who, by pointing out curious and interesting things that might otherwise escape notice is always raising the question of why and wherefore and sending the student to his textbook and reference manuals in a search for the answer. No book can replace such a man, but it should make an excellent supplement to his enthusiastic and inspiring preceptorship.

The Robert Jones Birthday Volume†

A COLLECTION of essays on orthopedic surgery dedicated to Sir Robert Jones on the occasion of his seventieth birthday. Naturally its chief interest will be to orthopedists but there is an especially interesting historical chapter which should have general interest.

Determinative Bacteriology‡‡

THE first edition of this work was published in August, 1923, and represented an initial effort on the part of the committee on Determinative Bacteriology of the Society of American Bacteriologists to place in the hands of students a detailed key for the identification of species based upon a classification of bacteria into families, tribes, and genera. A second edition of the work appeared in December, 1925, which embodied corrections of the previous work and amended descriptions of individual species. The work has become the standard authority in its field and of great practical value to all laboratories, students, and teachers engaged in bacteriological work.

The third edition of *Determinative Bacteriology* was published in January, 1930. This new volume represents a notable tome, greatly amplified with descriptions for some two hundred additional organisms not included in previous editions. These new organisms fall chiefly in genera Phytomonas, Flavobacterium, Pseudomonas, Lactobacillus, and Bacillus. Two additional tribes and four additional genera are also recognized. Of great interest is the division of Genus Eberthella placing the dysentery and the typhoid organisms in separate groups. Furthermore the new Manual has attempted to arrange the genera of tribe Bacteriae in a more logical manner and a new key for this tribe has been constructed.

In seven years, through three different editions, the Manual of *Determinative Bacteriology* has gained a place of preeminence as an authoritative work on bacteriologic classification and Professor Bergey and members of the committee are to be congratulated for this very important and most practical contribution to the science of bacteriology. No laboratory or scientific library is complete without it.

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*An Introduction to the Study of Human Anatomy. By Robert James Terry, A.B., M.D. Professor of Anatomy in Washington University. Cloth, pages 346. The Macmillan Company, New York, 1929.

†Oxford Medical Publications. The Robert Jones Birthday Volume. A collection of Surgical Essays. Cloth, pages 434. Humphrey Milford, Oxford University Press, American Branch, New York, 1928.

‡‡Bergey's Manual of Determinative Bacteriology. By David H. Bergey, Professor of Bacteriology, University of Pennsylvania. With an Index by Robert S. Breed. Ed. 3, cloth, 589 pages. Baltimore, The Williams and Wilkins Co.

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EDITORIALS

Poisoning From Snake Bite in the United States

FROM the earliest time, snakes have been of great interest to mankind. Perhaps in most cases this interest takes the form of an inborn revulsion, but to many their great interest lies in their biologic relation to man and the lower animals. Snakes and lizards represent one great order of living reptiles, of which the other three are turtles, crocodiles and a single species of the ancient group known as Rhynchocephalia. The other orders of this interesting group of vertebrates are all extinct, but they contained some of the largest animals ever on the face of the earth, such as the stegosaurs, dinosaurs and iethyosaurus.

The snakes have furnished zoologists with some of the most fascinating material on which many valuable investigations have been made, yielding answers to many of the interesting and significant problems in embryology, comparative anatomy, and phylogeny. The pharmacologist and physiologist both have found valuable material among the snakes, and study of their ven-

oms has made a brilliant chapter in their annals. But apparently not all the problems in herpetology have been solved, and the public health physician in certain localities, and physicians in general, are still confronted with many problems yet to be solved in the biologic relation of snakes to mankind. In these problems many laboratory men have been prominent; and so, recent work in the field should be of interest to pathologists.

The brilliant work of do Amaral in Brazil is now history, and the great benefit of the researches carried out in Sao Palo are well known. In 1926, under his direction, the Antivenin Institute of America was founded with a view to studying the conditions in the United States of America with regard to venomous snakes, and to prepare antitoxic serums against the bites of these snakes.

Fortunately there are but few species of poisonous snakes in the United States. All, with the exception of the coral snake, which has a limited distribution, belong to the same general group of pit vipers, and it was early found that a polyvalent antitoxic serum could be prepared that would be effective against all poisonous North American snakes except the coral snake, which rarely bites a human being. Our poisonous snakes belong to the Crotalidae and consist of the rattlesnake, the cotton-mouth moccasin and the copperhead. Venom extracted from these snakes can be used to prepare an antitoxic serum in horses, which is now generally dispensed. The polyvalent nature of the serum is of great practical value. This can be appreciated when one remembers that in the region of Panama and in South America, specific serums have to be administered according to the species of snake involved. It is interesting to note that the anticrotalin serum prepared from North American snakes does not neutralize the venom from Central American rattlesnakes such as *Crotalus terrificus*.

For the first time in the history of this country we have reliable data concerning the incidence of poisoning from snake bite, the effect of treatment, and the results, during a specific period of time. Thus, the report of Dr. R. H. Hutchinson of the Antivenin Institute of America for the year 1928 is of tremendous importance. Previous to this report, knowledge of such items was most unsatisfactory. Willson first estimated the number of cases in 1908; from his records he was able to collect 740 cases which dated back as far as 1843, and it is interesting to note that he concluded that poisonous snakes, in particular, rattlesnakes, were surely being exterminated and that their bites were becoming more and more infrequent. In this connection one might note that during the War of the Rebellion not a single case of snake bite was reported in the Medical and Surgical History of the War. Some time after 1908, Ditmars attempted to collect data on the cases of snake bite in the United States. From his data he estimated that at least 1000 cases of poisoning from snake bite occur in the United States each year, of which he believed 150 were fatal.

A total of 607 reports of cases of poisoning from snake bite which occurred in 1928, in continental United States, have been collected. Of these it is possible to analyze carefully 458. It further seems likely that these more carefully analyzed cases are the result of poisoning by ten species of snakes,

of which the copperhead leads, with 171 cases; the Texas rattler, with 100 cases, is next. The swamp rattler is given credit for only two cases. A comparatively small number of cases are reported to have been caused by the eastern diamond-back rattler, but a large number of bites were caused by the pigmy or ground rattler.

In studying the geographic distribution of poisoning by snake bite, one finds that it follows fairly closely the distribution of the snakes; however, certain discrepancies are to be noted. The distribution of reports of cases is influenced to a large measure by activity in the distribution of antivenin. Thus, the regions around certain cities, such as Houston and San Antonio, Texas, and Atlanta and Savannah, Georgia, become significant. In general, reports are few and scattered from the north Atlantic and north central states, with the exception of Pennsylvania, where most of the cases are due to bites by the copperhead. In the southeastern and Gulf states the distribution of cases shows that in Virginia and West Virginia, the chief offender is again the copperhead. In North Carolina, Georgia, Florida, Alabama, Mississippi, Louisiana and Arkansas, there is a somewhat different picture because of the inclusion of the cotton-mouth moccasin, pigmy rattler, and a few of the eastern diamond-back rattlers. In the Western and Southwestern states, the western diamond-back and the prairie rattlers are the predominating forms, with the former predominating in Texas and the latter predominating in Colorado. Even as far north as Montana, bites from the prairie rattler are recorded. In the Pacific Coast and Rocky Mountain states, the Pacific rattler is naturally the predominating form.

As would be expected, the seasonal distribution of cases follows the life history of the snakes. Few cases are reported before the end of April, which is correlated with the coming out from hibernation and the activity of the snakes during their period of feeding and breeding, which continues until December in the southern states. In the more northern latitudes, the activity of the snakes is limited to the summer season; cases do not appear as early in the year or continue as late. There is some difference in habits of the various species of snakes and with respect to time most reports are rather closely in accord with these specific habits in activity.

The distribution by sex of persons bitten reveals the anticipated result of about twice as many males as females. The distribution by age shows that 50 per cent of the bites occurred in persons under twenty years of age and that in children from two to five years of age, boys and girls were about equally represented.

The situation of the bite, in 57.8 per cent of cases, was on the lower extremities, and in 41 per cent of cases on the upper extremities. The circumstances under which the person was bitten determined to some extent the situation of the bite. It appears that 103 persons were bitten while picking up or lifting some object; thirty-seven, while reaching under some object; one hundred twenty, when they stepped on or too close to, or fell near, the snake; thirty-three while fishing or hunting; twenty-two while handling captive snakes, and the remainder were bitten under various circumstances.

The symptoms of poisoning from snake bite have been carefully analyzed for the year 1928. The appearance of symptoms in order of frequency was as follows: swelling, pain, weakness which appeared in the majority of cases, giddiness, respiratory difficulty, hemorrhage, weak pulse, vomiting, nausea, gangrene, ecchymosis, paralysis, increased pulse rate, unconsciousness, increased temperature, perspiration, and nervousness.

The most diversified forms of treatment are revealed by a study of these cases. The types of treatment may be subdivided as follows: (1) family or home remedies, including Indian folklore or superstitions; (2) simple measures of first aid, including the application of the tourniquet and the administration of stimulants; (3) chemical treatment of the wound (potassium permanganate is most generally used); (4) cauterization; (5) incision and suction, and (6) specific treatment with antivenin.

The best treatment has been summed up by Major R. E. Scott, who stated that in addition to the specific treatment with antivenin there are only three first-aid measures: first, placement of a tourniquet above the bite, just tight enough to obstruct the venous return. The tourniquet should be released at intervals of fifteen minutes for at least two minutes, to allow reestablishment of circulation. The sole purpose of the tourniquet is to diminish the rate at which the venom is allowed to enter the general circulation. Second, making of a crucial incision over the bite, sufficient to allow escape of blood serum. Third, mechanical suction, such as that supplied by a breast pump, to withdraw free venom at the site of the bite.

In the 607 cases reported, 175 patients were not treated with antivenin and in this group there were eighteen deaths (10.8 per cent). In most of these cases various kinds of medical and surgical treatment were employed. The species of snake involved varied considerably, as did the situation of the bite on the body. Death followed in each instance rather soon after the bite; the longest time was five days in one instance and the next longest time was only thirty hours. Death occurred in two hours in one instance. There were 433 cases in which the patients were given antivenin; in this group there were only thirteen deaths (3 per cent) and in 134 of the 433 cases, antivenin alone was depended on for relief of poisoning. In half of the cases in which death occurred, there was a delay of more than two hours in the administration of the antivenin, and in spite of the severity of the symptoms, antivenin was administered intravenously in only one case. Only four of the patients in this group who died received more than one dose of antivenin, and only one received three doses.

In a review of all of the cases in which antivenin was given, it was found that the serum was administered to 194 patients within two hours, and twenty-five patients received two or more doses. In the group of patients who died even after treatment with antivenin, a variety of snakes was involved, although the Texas rattler predominated. Death occurred three hours after the bite of the snake in one instance and life lasted forty-seven hours in the case in which the patient lived longest after being bitten.

The nature of the improvement is marked following the administration of antivenin. Improvement generally takes place within a few minutes after

its administration and certainly within a few hours. The relief of pain is one of the outstanding features of the effect of this serum, and the majority of cases reported indicate that the users were well satisfied with the effects observed. The question of how long after the bite antivenin is still effective probably cannot be answered, but it may be concluded that it is never too late to administer antivenin as long as the patient is alive.

In summarizing the treatment for snake bite one might offer the following suggestions for the victim:

1. Apply a ligature a few inches above the bite, release it for one minute every ten or fifteen minutes.

2. Administer antivenin if you have it with you.

3. Release the tourniquet.

4. If the bite has been inflicted by a large snake, in particular the Texas rattler or the Florida rattler, and if the symptoms are severe, supplement the treatment with incision and suction.

5. If antivenin is not at hand resort to incision and suction at once.

6. Obtain medical aid as quickly as possible.

7. Do not run or get overheated. Do not take any alcoholic stimulants. Do not inject potassium permanganate. Do not cauterize the bite. Do not depend on snake bite "cures."

The physician will have to use the same measures that have been outlined for the victim. He should repeat the injection of antivenin every one or two hours until the symptoms are diminished, and he should be prepared to resort to intramuscular or intravenous treatment. In cases in which there is marked shock, transfusion of blood or intravenous injection of solution of sodium chloride should be carried out. The wound should be taken care of by the usual aseptic measures.

One should remember that children require more antivenin in proportion to their weight than adults. The reason for this is a mathematical relation which exists between the weight of the body and the amount of venom which it can normally neutralize. The treatment of poisoning from snake bite that has been developed in the United States in the last two years, has been a distinct advance over the old forms of treatment, and it is further evidenced by this review that poisoning from snake bite is an important condition with which physicians should familiarize themselves.

—*T. B. M.*

Invitation to a Symposium on the Kidney in Health and Disease

To Be Held at the University of Minnesota Medical School

Minneapolis, Minnesota, July 7-18, 1930

Preliminary Program

In issuing this invitation to a symposium on the physiology and pathology of the kidney a few explanatory remarks may be appropriate. Attempts to facilitate the correlation of a great number of facts belonging to scattered compartments of human knowledge but all having a bearing on one subject, have become so numerous that this tendency might well be considered one of the characteristics of our times. The high degree of specialization that is associated with intensive scientific development makes such correlations particularly necessary—though they have always been needed and must, in fact, be of the same age as science itself.

We believe that in the field of internal medicine where this symposium belongs this kind of an attempt is not carried out often enough. Whether it ought to become a common occurrence will depend to no slight degree upon the benefit derived by the participants, passive as well as active. It may be pointed out as a characteristic of this symposium that it deals with a relatively small, well-defined subject. No attempt will be made to give a presentation of the complete accumulated knowledge of the kidney in health and disease. But, we will try to bring up for discussion those chapters of the anatomy, physiology, and pathology of the kidney where our knowledge has recently been extended in an important way together with other chapters where progress has been difficult to achieve, but where investigative efforts are intense.

Opening Address. Dr. A. J. Carlson, University of Chicago.
Form and Function of Renal Tubule. Dr. G. Carl Huber, University of Michigan.
The Comparative Anatomy of the Kidney. Dr. E. K. Marshall, Johns Hopkins Medical School, Baltimore, Md.
An Analysis of the Growth of the Human Kidney. Dr. R. E. Scammon, University of Minnesota.
Further Anatomic Considerations. Dr. C. M. Jackson, University of Minnesota.

After the foundation laid on the first day, the program will run along three parallel lines as follows:

A. Clinical Consideration of Kidney Diseases.
   Clinics on kidney disease, beginning Tuesday, July 8, at 8:00 A.M.
   Lectures at various other times.
B. Physiology of the Kidney.
   Lectures, beginning Tuesday, July 8, at 9:30 A.M.
C. Pathology of the Kidney.
   Lectures, beginning Tuesday, July 8, at 2:00 P.M.
D. Round table discussions every second day for the correlation of the material presented during the preceeding days.

Clinic. Prof. F. Volhard, Frankfort on the Main.
The Comparative Physiology of the Kidney. Dr. E. K. Marshall.
The Physiology of the Glomerulus. Dr. A. N. Richards, University of Pennsylvania.
General Pathologic Consideration—Glomerulonephritis. Dr. E. T. Bell, University of Minnesota.
The Physiology of the Tubules. Dr. H. L. White, Washington University, St. Louis, Mo.
The Physiology of the Tubules. Dr. R. N. Bieter.

Theory of Kidney Function—The Tubules—Threshold Substances. Dr. Poul B. Rehberg, The Zoophysiological Laboratory, University of Copenhagen.

Chronicity and Progression in Nephritis. Dr. Warfield T. Longcope, Johns Hopkins Hospital.

The Use of Urea and Creatinine in Testing the Function of Diseased Kidneys. Dr. Poul B. Rehberg.

The Phenolsulphonephthalein Test. Dr. L. G. Rowntree, The Mayo Clinic, Rochester, Minn.

Albuminuria and Plasma Proteins. Dr. H. Berglund, University of Minnesota.

The Colloids of Blood Plasma, the Hofmeister Series, and the Hydrogen-Ion Concentration. Dr. A. D. Hirschfelder, University of Minnesota.

Edema. Dr. L. Leiter, University of Chicago.

Edema. Dr. Poul B. Rehberg.

Hypertension. Statistical Considerations. Dr. Harold Diehl, University of Minnesota.

Hypertension. Dr. McN. Wetherby, University of Minnesota.

Hypertension. Dr. E. T. Bell.

The Retinal Changes in Bright's Disease and in Hypertension. Dr. H. P. Wagener, The Mayo Clinic, Rochester, Minn.

Uremia. Prof. F. Volhard.

Clinic on Hypertension. Dr. G. E. Fahr, University of Minnesota.

The Retinal Changes in Bright's Disease and in Hypertension (continued). Dr. H. P. Wagener.

Clinic on Hypertension. Prof. F. Volhard.

Fundamental Studies on Diuretics. Dr. R. N. Bieter.

The Effects of the Nerves on Glomerular Bloodflow. Dr. R. N. Bieter.

The Effect of the Nerves on Kidney Function. Dr. L. G. Rowntree.

Surgery of the Kidney in Bright's Disease. Prof. F. Volhard.

Clinic on Polycystic Kidney. Prof. F. Volhard.

The Clinical Uses of Diuretics. Dr. N. M. Kieth, The Mayo Clinic, Rochester, Minn.

The University asks all those who attend this symposium to register. There will be no registration fee.

The University will try to provide dormitory accommodations at a reasonable price during the symposium if registration is made before June 1.

Hotel accommodations will be arranged for if registration is made before the opening of the symposium.

All correspondence in regard to the symposium may be addressed to the Symposium, University Hospital, Minneapolis, Minnesota, or direct to Dr. Hilding Berglund, University Hospital, Minneapolis, Minnesota.

The final program, including a brief synopsis of each lecture, will be issued before June 1.

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Dallas, Texas
President

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Dr. F. W. Hartman, Detroit, Mich. 1928-9

ON TO DETROIT—NINTH ANNUAL CONVENTION

American Society of Clinical Pathologists

THE Ninth Annual Convention of the American Society of Clinical Pathologists will be held in Detroit, Michigan, June 20, 21, and 23, 1930, with headquarters in the Book-Cadillac Hotel. The Scientific and Business Sessions will take place in the Crystal Ball Room of the hotel and the Scientific and Commercial Exhibits in the adjoining Italian Garden.

The largest attendance in the history of the A. S. C. P. is anticipated. Detroit has been called the ideal convention city. Situated in the heart of the famous Great Lakes district it possesses all of the advantages of an industrially active world center together with the geographical characteristics of a summer resort. Detroit has a population of 1,790,000 persons and is so fortunately located that 70 per cent of the people of the United States are within an overnight's journey. The most important railroads of the nation run crack trains to Detroit; the finest steamers on the Great Lakes offer cool comfortable trips from Cleveland, Buffalo, Duluth, Chicago and intermediate points; bus lines from every important city and established airplane lines furnish the best in speedy travel, and super-highways that are without equal make motoring to Detroit a never-to-be-forgotten event. Detroit is a study

DR. KENNETH M. LYNCH
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DETROIT SKY LINE FROM THE RIVER

CADILLAC SQUARE

in paradoxes in that its artistic side is of as much importance as its industrial angle. One of the finest art centers in the nation has been developed in the city which emphasizes all of the divisions of the fine arts and offers opportunities to convention delegates and visitors for the study of some of the world's most beautiful paintings and pieces of sculpture. Canada is but a ten-minute ferry ride from Detroit. Windsor, across the river, is a place that

AMBASSADOR BRIDGE

DETROIT'S ART CENTER SHOWING DETROIT PUBLIC LIBRARY AT LEFT AND THE DETROIT INSTITUTE OF ARTS AT THE RIGHT (WOODWARD AVENUE CENTER)

everyone wants to visit. There one can stand on King George's territory and enjoy for a few hours the thrill of being in a foreign land. You will find there the spirit of Britain just as it is in London. The customs, the shops, the speech are a fragment of Piccadilly.

The Scientific Program is full to the brim with excellent papers. A wide variety of subjects should insure the enjoyment of all clinical pathologists no matter what their special genre is. The number of Scientific Exhibitors is greatly increased this year. A gold and a silver medal are offered as first and second award for the best scientific exhibits at the Convention. The Research Committee has planned a Symposium on Agranulocytosis which is expected

to arouse considerable general discussion. The Round Table Discussion on Friday evening, ever an enjoyable occasion, contains three pertinent subjects which will no doubt elicit the airing of opinions from all sections of the country. Presentation of medals will take place at the Annual Banquet on Saturday evening at which time we will also receive messages from the American College of Surgeons and the American Medical Association. The Ward Burdick Research Award will be presented to the winner chosen by the Research Committee by President J. H. Black. Dr. Black will also award the two Scientific Exhibits Medals to the recipients selected by the Scientific Exhibits Committee.

Book-Cadillac Hotel

Parke, Davis and Company of Detroit has invited the Fellows of the American Society of Clinical Pathologists in attendance at the convention to visit their laboratories at 1:15 P.M. on Friday and will serve luncheon to them at 2:15. There will be seen demonstrations of certain laboratory procedures and it is expected that this feature of our Convention will be very instructive and interesting.

The management of the Book-Cadillac Hotel has been very cooperative and assures us the best accommodations available. Members wishing reservations should communicate with the Secretary at once since all space is rapidly being taken up for the meeting of the American Medical Association.

You are therefore urged to lay aside the microscope long enough to attend this coming Convention, which from all indications promises to be our best ever.

ON TO DETROIT!

DR. FRANK W. HARTMAN
Detroit, Michigan
Chairman, Executive Committee

DR. A. H. SANFORD
Rochester, Minnesota
Chairman, Necrology Committee
Member, Executive Committee

DR. FREDERICK E. SONDERN
New York, N. Y.
Member, Executive Committee

DR. JOHN A. KOLMER
Philadelphia, Pa.
Chairman, Publication Committee
Member, Executive Committee

American Society of Clinical Pathologists

Program

Ninth Annual Convention

Detroit, Michigan

June 20, 21, and 23, 1930

FRIDAY MORNING, JUNE 20, 9 A.M.

Short Business Session

Scientific Program

The Diagnosis of Pregnancy by the Demonstration of the Hormone of the Anterior Lobe of the Hypophysis in the Urine. By H. L. Reinhart, M.D., and Ernest Scott, M.D., Columbus, Ohio.

Studies on Von Schilling Count. By Asher Yaguda, M.D., Newark, New Jersey.

Direct Calculation of the Volume and Hemoglobin Content of the Erythrocyte. A Comparison with Color Index, Volume Index and Saturation Index Determinations. By M. M. Wintrobe, M.D., New Orleans, Louisiana. (By invitation.)

The Kline Precipitation Reaction as a Routine Adjunct to the Complement-Fixation Test in the Serological Study of Syphilis. By Robert A. Kilduffe, M.D., Atlantic City, New Jersey.

Unexpected Findings in Unexpected Deaths. By Wm. J. Deadman, M.B., Hamilton, Canada.

Restraining the Unruly Patient while Performing Spinal Puncture. Motion Picture Demonstration. By H. A. Heise, M.D., Uniontown, Pennsylvania.

The Reaction of the Meninges to Therapeutic Serum. By Wm. M. Sheppe, M.D., Wheeling, West Virginia.

Coronary Occlusion. By Ernest Scott, M.D., and Mary K. Helz, Columbus, Ohio. To be read by title.

FRIDAY AFTERNOON, JUNE 20

Visit to Laboratories of Parke, Davis and Company. Laboratory Demonstrations, 1:15 P.M., Luncheon, 2:15 P.M.

Symposium on Agranulocytosis

Hematological Aspects of Agranulocytosis and Other Diseases Accompanied by Extreme Leucopenia. By Nathan Rosenthal, M.D., New York, N. Y.

Agranulocytic Syndromes. By E. L. Miloslavich, M.D., and Francis D. Murphy, M.D., Milwaukee, Wisconsin.

Report of Case with Recovery. Lantern Slides. By L. W. Larson, M.D., Bismarck, North Dakota.

Gastric Manifestations of Aleukemic Leukemia. By Kano Ikeda, M.D., St. Paul, Minnesota.

FRIDAY EVENING, JUNE 20, 7:30 P.M.

Round Table Discussion

Development of an Autopsy Service. By Asher Yaguda, M.D., Newark, New Jersey.

The Development of Local Pathological Societies as Component Parts of the American Society of Clinical Pathologists. By Wm. M. Sheppe, M.D., Wheeling, West Virginia.

Is It Ethical for a Clinical Pathologist to Advertise? By J. J. Moore, M.D., Chicago, Illinois.

SATURDAY MORNING, JUNE 21, 9 A.M.

Hemorrhage with Sudden Death in Tracheo-Bronchial Lymph Node Tuberculosis in Adults. By H. A. Callis, M.D., Tuskegee, Alabama.

Additional Observations on Isolating Tubercle Bacilli. By H. J. Corper, M.D., and Nao Uyei, Ph.D., Denver, Colorado.

The Use of the Photo-Electric Effect in Clinical Pathology. By Wm. G. Exton, M.D., Newark, New Jersey.

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Columbus, Ohio
Member, Board of Censors

The Electromotive Thermometer: An Instrument Equipped with Thermocouples for the Measurement of Skin and Intramural Temperatures. By Charles Sheard, Ph.D., Rochester, Minnesota.

Methods and Effects of Increasing the Urinary Constituents in the Body. By Frank W. Hartman, M.D., Detroit, Michigan.

Experimental Fat Necrosis in Various Vertebrates. By M. Pinson Neal, M.D., and Max M. Ellis, Ph.D., Columbia, Missouri.

Vascular Injury by the Pneumococcus and Its Relation to Red Hepatization in Lobar Pneumonia. By Theodore J. Curphey, M.D., New York, N. Y.

Gas Bacillus Infection in Civil Life. By Walter E. King, M.D., Detroit, Michigan.

SATURDAY AFTERNOON, JUNE 21, 2 P.M.

Gastrointestinal Disturbances in Endocrine Diseases. By Michael G. Wohl, M.D., Philadelphia, Pa.

Pathogenesis of Goitre. By B. Markowitz, M.D., Bloomington, Illinois.

Thyroid—The Inflammatory Nature of Nodular Goitre as a Chronic Thyroiditis. By Robert A. Keilty, M.D., Washington, D. C.

The Present Status of Our Knowledge of Cancer. By William Carpenter MacCarty, M.D., Rochester, Minnesota.

Stains in the Microscopic Diagnosis of Malignancy. By Charles Geschickter, M.D., Baltimore, Maryland. (By invitation.)

The Diagnosis of Border-Line Breast Tumors. By Joseph C. Bloodgood, M.D., Baltimore, Maryland. (By invitation.)

Some Unusual Pathologic Tissues. By Philip Hillkowitz, M.D., Denver, Colorado.

SATURDAY EVENING, JUNE 21, 7:30 P.M.

Annual Banquet

Presidential Address. By J. H. Black, M.D., Dallas, Texas.

Measuring the Efficiency of the Clinical Laboratory. By Malcolm T. MacEachern, M.D., Associate Director of the American College of Surgeons, Chicago, Illinois.

Clinical Laboratories and Medical Progress. By N. P. Colwell, M.D., Secretary of the Council on Medical Education and Hospitals of the American Medical Association, Chicago, Illinois.

Presentation of Scientific Exhibits Medals. By President J. H. Black, M.D.

Presentation of the Ward Burdick Research Award. By President J. H. Black, M.D.

MONDAY, JUNE 23

Business Session

Call to order.

Reading of Minutes.

Unfinished Business.

Reports of Committees:

Executive Committee—Frank W. Hartman, M.D., Detroit, Michigan, Chairman.

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Editorial Committee—T. B. Magath, M.D., Rochester, Minnesota, Chairman.

Research Committee—Alvin G. Foord, M.D., Buffalo, New York, Chairman.

Public Relations Committee—F. B. Johnson, M.D., Charleston, S. C., Chairman.

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Board of Registry of Technicians—Philip Hillkowitz, M.D., Denver, Colorado, Chairman.

Scientific Exhibits Committee—Clarence I. Owen, M.D., Detroit, Mich., Chairman.

Necrology Committee—A. H. Sanford, M.D., Rochester, Minnesota, Chairman.

Report of the Board of Censors—Election of New Members.
F. H. Lamb, M.D., Davenport, Iowa, Chairman.

New Business.

Report of the Nominating Committee—Nomination of Officers.

Election of Officers.

Induction of Officers.

Adjournment.

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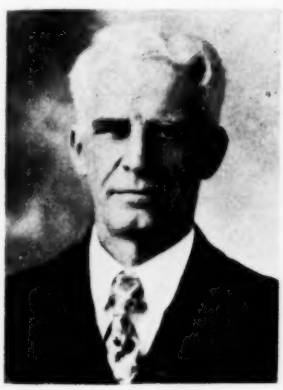

DR. F. B. JOHNSON
Charleston, S. C.
Chairman, Public Relations Committee

DR. CLARENCE I. OWEN
Detroit, Michigan
Chairman, Scientific Exhibits Committee

Scientific Exhibits

An Exhibit of Preserved Specimens and Photographs Illustrating the Changes Produced in the Ovaries of Experimental Animals as the Result of the Injection of Urine Containing Pituitary Hormone. By Ernest Scott, M.D., Columbus, Ohio.

Microscopic Slide Precipitation Tests for Syphilis of Unheated Serum, Heated Serum, Defibrinated Finger Blood and Unconcentrated Spinal Fluid. By B. S. Kline, M.D., Cleveland, Ohio.

Photographs and Microphotographs Illustrating Chronic Thyroiditis. By Robert A. Keilty, M.D., Washington, D. C.

The Aschheim-Zondek Pregnancy Test. By E. R. Mugrage, M.D., and Rodney H. Jones, M.D., Denver, Colorado.

Coccidioidal Granuloma. By W. T. Cummins, M.D., San Francisco, California, Joseph K. Smith, M.D., Bakersfield, California and C. H. Halliday, Baltimore, Maryland.

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Dr. Vaughan in His Laboratory, 1914

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Dedicate with Deep Affection
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To the Memory of its First
Editor-in-chief

VICTOR CLARENCE VAUGHAN

To Whose Initiative, Enthusiasm and Untiring Zeal
The Journal
Owes its Present Prestige and Usefulness
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It may not be given to us to solve the riddle of the universe but this need not deter us from doing the duty that lies so plainly before us and the most exalted privilege that comes to man is to labor for the uplift of his race.

—*Victor C. Vaughan*